CUE 02 0004

For EPA Use Only
ID#______

Worksheet 1. Contact and Methyl Bromide Request Information

The following information will be used to determine the amount of methyl bromide requested and the contact person for this request. It is important that we know whom to contact in case we need additional information during the review of the application.

1. **Location**
(Enter the state, region, or county. Provide more detail about the location if relevant to the feasibility of alternatives to methyl bromide.)

Michigan, USA

2. **Crop/commodity**
(Include all crops/commodities that benefit from the application of methyl bromide in a fumigation cycle. A fumigation cycle is the period of time between methyl bromide fumigations.)

This application is for solanaceous crops (1 crop/fumigation cycle). Solanaceous crops include tomato, pepper, and eggplant.

3. **Climate**
(Individual users should enter their climate zone designation by reviewing the U.S. climate zone map. If a consortium is submitting this application, please indicate the estimated percentage of consortium users in each climate zone. This map is located at the end of this workbook or it can be reviewed online at http://www.usna.usda.gov/ Hardzone/ushzmap.html).

All users are located in zone 5B (average annual minimum temperature -10 to -15 F).

4. **Soil type** Check the box(es) for the soil types and percent organic matter that apply to your area. If a consortium is submitting this application, please indicate the estimated percentage of consortium users in each soil type.

Soil Type:	**Light**	X	**Medium**	X	**Heavy**	
Organic Matter:	**0 to 2%**	25	**2 to 5 %**	75	**over 5%**	

5. **Other geographic factors that may affect crop/commodity yield (e.g., water table).**

6. **Consortium name** Michigan solanaceous crop (tomato, eggplant, pepper) growers

Specialty (check one)

7. **Contact name** Dr. Mary Hausbeck

agronomic X

economic ____

8. **Address** Mich. State Univ., Dept. of Plant Pathology
140 Plant Biology Lab
E. Lansing, MI, USA 48824-1312

9. **Daytime phone** 517-355-4534

10. **FAX** 517-353-9704

11. **E-mail** hausbec1@msu.edu

List an additional contact person if available.

Specialty (check one)

12. **Contact name** Barbara Dartt, DVM, MS

agronomic ____

economic X

13. **Address** Salisbury Management Services, Inc.
2487 S. Michigan, P.O. Box 10
Eaton Rapids, MI, USA 48827-0010

14. **Daytime phone** 517-663-5600

15. **FAX** 517-663-5608

16. **E-mail** bdartt@salisbury-management.com

Worksheet 1. Contact and Methyl Bromide Request Information

For EPA Use Only
ID#

17. How much active ingredient (ai) of methyl bromide are you requesting for 2005? 115,408 **lbs.**

If a consortium is submitting this application, the data for question 17 and 17a. should be the total for the consortium.

In the question below, area is defined as follows for each user: acres for growers, cubic feet for post harvest operations, and square feet for structural applications.

17a. How much area will this be applied to? Please list units. 2,687 acres **units**

18. Are you requesting methyl bromide for additional years beyond 2005? **Yes** X **No** ____

18a. If yes, please list year and quantity active ingredient (ai) of methyl bromide requested in the table below and explain why you need authorization for multiple years.

Additional time is needed to facilitate testing of potential alternatives for crop safety, pathogen efficacy, and incorporation into commercial production systems. Also, we anticipate that additional growing seasons are needed for demonstration plots with grower cooperators.

If a consortium is submitting this application, the data below should be the total for the consortium.

In the table below, **area is defined** as follows for each user: acres for growers, cubic feet for post harvest operations, and square feet for structural applications.

Year	Quantity ai (lb.) of Methyl Bromide	Area to be Treated	Unit of Area Treated
2006	113,230	2,636	acres
2007	108,875	2,535	acres

19. Target Pest(s) or Pest Problem(s):
(Be as specific as possible about the species or classes of pests relevant to the feasibility of alternatives.)

Soil-borne fungi that cause crown, root, and fruit rot, including *Phytophthora capsici* (primary problem), *Verticillium* spp., and Fusarium oxysporum f.sp. *lycopersicae*.

20. If applying as a consortium for many users of methyl bromide, please define a *representative user*. Define exactly, issues such as size of the operation (acres treated with methyl bromide for growers, cubic feet for post-harvest operations, and square feet for structural applications), whether the representative user owns or rents the land or operation, intensity of methyl bromide use (treat regularly or only when pest reaches a threshold), pest pressure, etc.

A representative user employs raised beds, black plastic, and trickle irrigation. The fumigant is applied preplant. The user owns the land and operation. The user utilizes rotation, but due to pathogen longevity the problem is treated regularly. The user grows for the fresh market industry, and requires earliness and blemish-free produce for several days postharvest.

20a. Explain why this user represents the typical user in the consortium.

The typical user grows for the fresh market industry. Raised beds are used to decrease *Phytophthora* problems. Trickle irrigation is used to prevent blossom end rot, and black plastic for weed control.

Worksheet 2-A. Methyl Bromide - Use 1997-2000

If a consortium is submitting this application, all data should reflect the **actual** data for the consortium.	
Col A: Formulation of Methyl Bromide	Enter the appropriate data in Col B-M for each formulation, if known, and/or the totals and averages for all formulations. If you enter only the total and averages for all formulations in the last row of the table, please describe in the comments section the formulations typically used, or the approximate proportions of the formulations used.
Col B, E, H, K: Actual Area Treated	Enter the total actual area treated. Note: This number should be the total actual area treated by the individual user or total actual area for the entire consortium, for the year indicated.
Col C, F, I, L: Actual Total lbs. ai of Methyl Bromide Applied	Enter the actual total pounds active ingredient (ai) of methyl bromide applied. Note: This number should be the total pounds ai applied by the individual user or the entire consortium, for the year indicated.
Col D, G, J, M: Actual Average lbs. ai Applied per Area	The average application rates in pounds ai of methyl bromide per area are automatically calculated from the previous 2 columns.

Area is defined below as follows for each user: acres for growers, cubic feet for post-harvest operations, and square feet for structural applications.

A	B	C	D	E	F	G	H	I	J	K	L	M
Formulation of Methyl Bromide	**1997**			**1998**			**1999**			**2000**		
	Total Actual Area Treated	**Actual Total lbs. ai of Methyl Bromide Applied**	**Average lbs. ai Applied per Area**	**Total Actual Area Treated**	**Actual Total lbs. ai of Methyl Bromide Applied**	**Average lbs. ai Applied per Area**	**Total Actual Area Treated**	**Actual Total lbs. ai of Methyl Bromide Applied**	**Average lbs. ai Applied per Area**	**Total Actual Area Treated**	**Actual Total lbs. ai of Methyl Bromide Applied**	**Average lbs. ai Applied per Area**
over 95% methyl bromide												
75% methyl bromide, 25% chloropicrin												
67% methyl bromide, 33% chloropicrin	1054	113230	107.428843	1013	108875	107.477789	1013	108875	107.477789	1156	124118	107.368512
50% methyl bromide, 50% chloropicrin												
__% methyl bromide, __% chloropicrin												
__% methyl bromide, __% chloropicrin												
All formulations of methyl bromide	1054	113230	107.428843	1013	108875	107.477789	1013	108875	107.477789	1156	124118	107.368512

Comments:
Actual area treated is in acres. Applications were made under plastic with an average bed width of 24".

Application for Critical Use Exemption of Methyl Bromide
for Use in 2005 in the United States

Economic Summary:

Budgets representative of eight Michigan fresh market vegetable crops were constructed using grower focus groups. These budgets were compared to the alternative revenue and cost structures present if no methyl bromide were utilized. In the case of all eight crops, use of methyl bromide generates higher profits than production without this fumigant.

Application for Critical Use Exemption of Methyl Bromide for Use in 2005 in the United States

Worksheet 2-D. Methyl Bromide - Use and Costs for 2001

In Michigan fresh market vegetable crops, methyl bromide (MB) is utilized as a component of a plasticulture system. Fields are prepared with a minimal amount of traditional tillage using moldboard plows, tandem discs and tractors of 100-140 horsepower. Following this preparation, a tractor (100 hp) pulls a piece of equipment called a bedder or plastic layer. This equipment simultaneously forms beds, lays plastic and drip tube and injects MB. This equipment can usually bed about 1 acre per hour and utilizes a crew of 4 laborers and an equipment operator. The operating and ownership costs of running this equipment and paying the crew were calculated to be $98 per acre. It is not possible to separate the application of MB from the portions of the plasticulture system.

Worksheet 2-C. Methyl Bromide - Crop/Commodity Yield and Gross Revenue 2001

Eight vegetable crops are included in this application. They are:

Curcubit Crops
- Cantaloupe
- Cucumber
- Hard Squash
- Watermelon
- Zucchini

Solanaceous Crops
- Eggplant
- Tomato
- Green Pepper

Price data for these crops were averaged across quality grades and seasonal differences. Use of MB does facilitate earlier harvests because it has a shorter spring waiting period than alternatives. Methyl bromide also supports growth of a healthier, more vigorous plant. On average, crops produced with methyl bromide bring a higher price because they can be marketed in more timely manner, taking advantage of early high prices. In addition, a larger percentage of product can be marketed in quality and size grades that bring a higher price.

Worksheet 2-E. Methyl Bromide - *Other* Operating Costs for 2001

Methodology and Assumptions for Budgets Provided in Place of Worksheet 2-E.

The budgets for the eight crops included in this application were developed using grower focus groups with a good knowledge of the industry and good field, enterprise, and financial records. The process was initiated by defining individual production systems representative of Michigan. Subsequently, both the sequence of decisions and the information necessary to make these key decisions was collected. This process resulted in a list of inputs and input prices that were then translated into costs. These costs were verified against grower records. These budgets reproduce, as completely as possible, all costs incurred by growers.

Below are comments about the methods used in particular areas of each budget.

Costs of Capital Services (Buildings, Machinery, and Equipment)
Estimating the annual cost of using buildings, machinery, equipment and other assets is a challenge in cost of production studies. Buildings, machinery and services were priced to the enterprise on a "custom" basis. Further, services such as land preparation were priced to the enterprise as a "bundled" service/task reflecting both the machinery and labor components of the service.

This approach requires some judgment because costs such as buildings to house machinery and equipment, the farm shop, and labor used in maintenance of machinery and equipment must be included in the "custom fee" as well as the "depreciation and interest" on the machinery and equipment. The fact that this custom fee approach was used does not imply that custom operators did all the tasks. It simply means the tasks are priced to the enterprise as if a custom operator had completed them. The services may well have been provided by the "machinery services enterprise" of the farm. As a double check, members of the focus group attempted to compare the aggregate custom fee costs to those based on their accounting records which included labor, custom fees, and depreciation and interest on buildings, machinery, and equipment. Custom fees were also double-checked against survey information when available.

Worksheet 2-F. Methyl Bromide Fixed and Overhead Costs in 2001

Fixed costs including management and supervision, insurance and other overhead were allocated equally across an entity's total vegetable acres. In the case of management and labor, adjustments were made to account for increased time demands of crops with a more complex biological or production cyce.

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ID#

Worksheet 2-B. Methyl Bromide - Crop/Commodity Yield and Gross Revenue 1997-2000

If a consortium is submitting this application, the data for this table should reflect the **actual averages** for the consortium.	
The purpose of this worksheet is to estimate the gross revenue for 1997 - 2000 when using methyl bromide. Post-harvest and structural users may work with EPA to modify this	
Col. A: Year	Be sure to enter the year. Use as many rows as needed for each year for all the crops/commodities in the fumigation cycles from 1997 to
Col. B: Crop/Commodity	Enter all crops/commodities that benefit from methyl bromide in each fumigation cycle. (For example, if normally methyl bromide is applied If someone other than the applicant benefits from the application of methyl bromide in the fumigation cycle and you do not have the
Col. C: Unit of	Enter the unit of measurement for each crop/commodity.
Col. D: Crop/Commodity Yield	Enter the number of units of crop/commodities produced per area.
Col. E: Price	Enter the average prices received by the users for the year and crop/commodity indicated (1997-2000).
Col. F: Revenue	This number is calculated automatically using the values you entered in Cols. D and E. You may override the formula to enter a different
Total Revenue for 1997-2000	Enter the total revenue per year by adding the revenue for all crops for that year.
Average Revenue per Year:	The average revenue per year is calculated automatically using the summary data you enter for each year.
Area is defined below as follows for each user: acres for growers, cubic feet for post-harvest operations, and square feet for structural applications.	

A	B	C	D	E	F
Year Methyl Bromide was Applied	**Crop/Commodity**	**Unit of Crop/Commodity** (e.g., pounds, bushels)	**Crop/Commodity Yield** (Units per area)	**Price** (per unit of crop/commodity)	**Revenue** (per area)
1997	Tomatoes	25 lb. Boxes	1780	$ 9.73	$ 17,320.77
1998	Tomatoes	25 lb. Boxes	1765	$ 9.62	$ 16,980.85
1999	Tomatoes	25 lb. Boxes	1880	$ 10.18	$ 19,140.88
2000	Tomatoes	25 lb. Boxes	1560	$ 9.57	$ 14,922.86
2001					$ 0.00
					$ 0.00
					$ 0.00
					$ 0.00
					$ 0.00
					$ 0.00
					$ 0.00
					$ 0.00
				Total Revenue for 1997	$ 17,320.77
				Total Revenue for 1998	$ 16,980.85
				Total Revenue for 1999	$ 19,140.88
				Total Revenue for 2000	$ 14,922.86
				Average Revenue Per Year	$ 17,091.34

Comments:

OMB Control # 2060-0482

Michigan Tomato
WITH Methyl Bromide

	Quantity	Unit	Price per Unit	Total per Acre
Revenue		25 lb		
Tomatoes	1500	boxes	$ 9.93	$ 14,893
Total Revenue				**$ 14,893**
Expenses				
Field Preparation				
Cover Crop (Materials, Machinery & Labor)				$ 13
Field tillage				$ 39
Soil Test				$ 2
Lime & Application				$ 13
Fertilizer (Materials)				$ 212
Apply & Incorporate Fertilizer (Machinery & Labor)				$ 14
Plastic & Drip Tape (Materials)				$ 284
Lay Plastic & Drip Tape (Machinery & Labor)				$ 98
Fumigate (Materials)				$ 341
Herbicide, Insecticide & Fungicide (Materials)				$ 425
Apply Herbicide, Insecticide & Fungicide (Mach & Lab)				$ 54
Plant & Grow				
Transplants				$ 600
Planting				$ 75
Staking & Tieing (Machinery, Labor & Materials)				$ 627
Pruning				$ 120
Drip Irrigate & Fertigate (Labor, Materials & Electricity)				$ 275
Scouting & Lab Work				$ 30
Field Maintenance - Driveways & Mowing (Mach & Lab)				$ 7
Harvest				
Pick Crop (Field to Packing Shed)				$ 1,800
Grading & Packing (Includes shipping containers)				$ 4,500
Shipping (Includes Materials, Machinery & Labor)				$ 750
Sales & Marketing	9%	of gross		$ 1,340
Field Clean-up				$ 112
Management & Supervision				$ 125
Interest on Operating Capital		8%		$ 298
Land Rent				$ 200
Insurance				$ 8
Other Overhead (Professional Fees, Education & Travel, etc)				$ 10
Total Expenses				**$ 12,372**
PROFIT				**$ 2,521**

Michigan Tomato
WITHOUT Methyl Bromide

	Quantity	Unit	Price per Unit	Total per Acre
Revenue		25 lb		
Tomatoes	975	boxes	$ 6.95	$ 6,776
Total Revenue				**$ 6,776**
Expenses				
Field Preparation				
Cover Crop (Materials, Machinery & Labor)				$ 13
Field tillage				$ 39
Soil Test				$ 2
Lime & Application				$ 13
Fertilizer (Materials)				$ 212
Apply & Incorporate Fertilizer (Machinery & Labor)				$ 14
Plastic & Drip Tape (Materials)				$ 284
Lay Plastic & Drip Tape (Machinery & Labor)				$ 98
Fumigate (Materials)				
Herbicide, Insecticide & Fungicide (Materials)				$ 470
Apply Herbicide, Insecticide & Fungicide (Mach & Lab)				$ 60
Plant & Grow				
Transplants				$ 600
Planting				$ 75
Staking & Tieing (Machinery, Labor & Materials)				$ 627
Pruning				$ 120
Drip Irrigate & Fertigate (Labor, Materials & Electricity)				$ 275
Scouting & Lab Work				$ 30
Field Maintenance - Driveways & Mowing (Mach & L:				$ 7
Harvest				
Pick Crop (Field to Packing Shed)				$ 1,170
Grading & Packing (Includes shipping containers)				$ 2,925
Shipping (Includes Materials, Machinery & Labor)				$ 488
Sales & Marketing	9%	of gross		$ 610
Field Clean-up				$ 112
Management & Supervision				$ 125
Interest on Operating Capital		8%		$ 136
Land Rent				$ 200
Insurance				$ 8
Other Overhead (Professional Fees, Education & Travel, etc)				$ 10
Total Expenses				**$ 8,722**
PROFIT				**$ (1,946)**

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Worksheet 2-B. Methyl Bromide - Crop/Commodity Yield and Gross Revenue 1997-2000

If a consortium is submitting this application, the data for this table should reflect the **actual averages** for the consortium.	
The purpose of this worksheet is to estimate the gross revenue for 1997 - 2000 when using methyl bromide. Post-harvest and structural users may work with EPA to modify this	
Col. A: Year	Be sure to enter the year. Use as many rows as needed for each year for all the crops/commodities in the fumigation cycles from 1997 to
Col. B: Crop/Commodity	Enter all crops/commodities that benefit from methyl bromide in each fumigation cycle. (For example, if normally methyl bromide is applied If someone other than the applicant benefits from the application of methyl bromide in the fumigation cycle and you do not have the
Col. C: Unit of	Enter the unit of measurement for each crop/commodity.
Col. D: Crop/Commodity Yield	Enter the number of units of crop/commodities produced per area.
Col. E: Price	Enter the average prices received by the users for the year and crop/commodity indicated (1997-2000).
Col. F: Revenue	This number is calculated automatically using the values you entered in Cols. D and E. You may override the formula to enter a different
Total Revenue for 1997-2000	Enter the total revenue per year by adding the revenue for all crops for that year.
Average Revenue per Year:	The average revenue per year is calculated automatically using the summary data you enter for each year.
Area is defined below as follows for each user: acres for growers, cubic feet for post-harvest operations, and square feet for structural applications.	

A	B	C	D	E	F
Year Methyl Bromide was Applied	**Crop/Commodity**	**Unit of Crop/Commodity** (e.g., pounds, bushels)	**Crop/Commodity Yield** (Units per area)	**Price** (per unit of crop/commodity)	**Revenue** (per area)
1997	Tomatoes	25 lb. Boxes	1780	$ 9.73	$ 17,320.77
1998	Tomatoes	25 lb. Boxes	1765	$ 9.62	$ 16,980.85
1999	Tomatoes	25 lb. Boxes	1880	$ 10.18	$ 19,140.88
2000	Tomatoes	25 lb. Boxes	1560	$ 9.57	$ 14,922.86
2001					$ 0.00
					$ 0.00
					$ 0.00
					$ 0.00
					$ 0.00
					$ 0.00
					$ 0.00
					$ 0.00
				Total Revenue for 1997	$ 17,320.77
				Total Revenue for 1998	$ 16,980.85
				Total Revenue for 1999	$ 19,140.88
				Total Revenue for 2000	$ 14,922.86
				Average Revenue Per Year	$ 17,091.34

Comments:

Michigan Tomato
WITH Methyl Bromide

	Quantity	Unit	Price per Unit	Total per Acre
Revenue		25 lb		
Tomatoes	1500	boxes	$ 9.93	$ 14,893
Total Revenue				**$ 14,893**
Expenses				
Field Preparation				
Cover Crop (Materials, Machinery & Labor)				$ 13
Field tillage				$ 39
Soil Test				$ 2
Lime & Application				$ 13
Fertilizer (Materials)				$ 212
Apply & Incorporate Fertilizer (Machinery & Labor)				$ 14
Plastic & Drip Tape (Materials)				$ 284
Lay Plastic & Drip Tape (Machinery & Labor)				$ 98
Fumigate (Materials)				$ 341
Herbicide, Insecticide & Fungicide (Materials)				$ 425
Apply Herbicide, Insecticide & Fungicide (Mach & Lab)				$ 54
Plant & Grow				
Transplants				$ 600
Planting				$ 75
Staking & Tieing (Machinery, Labor & Materials)				$ 627
Pruning				$ 120
Drip Irrigate & Fertigate (Labor, Materials & Electricity)				$ 275
Scouting & Lab Work				$ 30
Field Maintenance - Driveways & Mowing (Mach & Lab)				$ 7
Harvest				
Pick Crop (Field to Packing Shed)				$ 1,800
Grading & Packing (Includes shipping containers)				$ 4,500
Shipping (Includes Materials, Machinery & Labor)				$ 750
Sales & Marketing	9%	of gross		$ 1,340
Field Clean-up				$ 112
Management & Supervision				$ 125
Interest on Operating Capital		8%		$ 298
Land Rent				$ 200
Insurance				$ 8
Other Overhead (Professional Fees, Education & Travel, etc)				$ 10
Total Expenses				**$ 12,372**
PROFIT				**$ 2,521**

Michigan Tomato
WITHOUT Methyl Bromide

	Quantity	Unit	Price per Unit	Total per Acre
Revenue		25 lb		
Tomatoes	975	boxes	$ 6.95	$ 6,776
Total Revenue				**$ 6,776**
Expenses				
Field Preparation				
Cover Crop (Materials, Machinery & Labor)				$ 13
Field tillage				$ 39
Soil Test				$ 2
Lime & Application				$ 13
Fertilizer (Materials)				$ 212
Apply & Incorporate Fertilizer (Machinery & Labor)				$ 14
Plastic & Drip Tape (Materials)				$ 284
Lay Plastic & Drip Tape (Machinery & Labor)				$ 98
Fumigate (Materials)				
Herbicide, Insecticide & Fungicide (Materials)				$ 470
Apply Herbicide, Insecticide & Fungicide (Mach & Lab)				$ 60
Plant & Grow				
Transplants				$ 600
Planting				$ 75
Staking & Tieing (Machinery, Labor & Materials)				$ 627
Pruning				$ 120
Drip Irrigate & Fertigate (Labor, Materials & Electricity)				$ 275
Scouting & Lab Work				$ 30
Field Maintenance - Driveways & Mowing (Mach & L				$ 7
Harvest				
Pick Crop (Field to Packing Shed)				$ 1,170
Grading & Packing (Includes shipping containers)				$ 2,925
Shipping (Includes Materials, Machinery & Labor)				$ 488
Sales & Marketing	9%	of gross		$ 610
Field Clean-up				$ 112
Management & Supervision				$ 125
Interest on Operating Capital		8%		$ 136
Land Rent				$ 200
Insurance				$ 8
Other Overhead (Professional Fees, Education & Travel, etc)				$ 10
Total Expenses				**$ 8,722**
PROFIT				**$ (1,946)**

Worksheet 2-B. Methyl Bromide - Crop/Commodity Yield and Gross Revenue 1997-2000

If a consortium is submitting this application, the data for this table should reflect the **actual averages** for the consortium.	
The purpose of this worksheet is to estimate the gross revenue for 1997 - 2000 when using methyl bromide. Post-harvest and structural users may work with EPA to modify this	
Col. A: Year	Be sure to enter the year. Use as many rows as needed for each year for all the crops/commodities in the fumigation cycles from 1997 to
Col. B: Crop/Commodity	Enter all crops/commodities that benefit from methyl bromide in each fumigation cycle. (For example, if normally methyl bromide is applied If someone other than the applicant benefits from the application of methyl bromide in the fumigation cycle and you do not have the
Col. C: Unit of	Enter the unit of measurement for each crop/commodity.
Col. D: Crop/Commodity Yield	Enter the number of units of crop/commodities produced per area.
Col. E: Price	Enter the average prices received by the users for the year and crop/commodity indicated (1997-2000).
Col. F: Revenue	This number is calculated automatically using the values you entered in Cols. D and E. You may override the formula to enter a different
Total Revenue for 1997-2000	Enter the total revenue per year by adding the revenue for all crops for that year.
Average Revenue per Year:	The average revenue per year is calculated automatically using the summary data you enter for each year.
Area is defined below as follows for each user: acres for growers, cubic feet for post-harvest operations, and square feet for structural applications.	

A	B	C	D	E	F
Year Methyl Bromide was Applied	**Crop/Commodity**	**Unit of Crop/Commodity** (e.g., pounds, bushels)	**Crop/Commodity Yield** (Units per area)	**Price** (per unit of crop/commodity)	**Revenue** (per area)
1997	Eggplants	1 1/9 Bushels	1500	$ 8.45	$ 12,675.82
1998	Eggplants	1 1/9 Bushels	1500	$ 10.49	$ 15,741.76
1999	Eggplants	1 1/9 Bushels	1500	$ 8.69	$ 13,038.46
2000	Eggplants	1 1/9 Bushels	1500	$ 9.57	$ 14,357.14
2001					$ 0.00
					$ 0.00
					$ 0.00
					$ 0.00
					$ 0.00
					$ 0.00
					$ 0.00
					$ 0.00
				Total Revenue for 1997	$ 12,675.82
				Total Revenue for 1998	$ 15,741.76
				Total Revenue for 1999	$ 13,038.46
				Total Revenue for 2000	$ 14,357.14
				Average Revenue Per Year	$ 13,953.30

Comments:

Michigan Eggplant
WITH Methyl Bromide

	Quantity	Unit	Price per Unit		Total per Acre
Revenue					
Eggplant	1500	1 1/9 bu boxes	$ 8.49	$	12,742
Total Revenue				$	12,742
Expenses					
Field Preparation					
Cover Crop (Materials, Machinery & Labor)				$	13
Field tillage				$	39
Soil Test				$	2
Lime & Application				$	13
Fertilizer (Materials)				$	100
Apply & Incorporate Fertilizer (Machinery & Labor)				$	34
Plastic & Drip Tape (Materials)				$	284
Lay Plastic & Drip Tape (Machinery & Labor)				$	98
Fumigate (Materials)				$	341
Herbicide, Insecticide & Fungicide (Materials)				$	165
Apply Herbicide, Insecticide & Fungicide (Mach & Lab)				$	78
Plant & Grow					
Transplants				$	848
Planting				$	75
Staking & Tieing (Machinery, Labor & Materials)				$	258
Cultivate & Hoe				$	94
Drip Irrigate & Fertigate (Labor, Materials & Electricity)				$	267
Scouting & Lab Work				$	30
Field Maintenance - Driveways & Mowing (Mach & Lab)				$	7
Harvest					
Pick Crop (Field to Packing Shed)				$	1,800
Grading & Packing (Includes shipping containers)				$	4,485
Shipping (Includes Materials, Machinery & Labor)				$	750
Sales & Marketing	9%	of gross		$	1,147
Field Clean-up				$	112
Management & Supervision				$	75
Interest on Operating Capital		8%		$	255
Land Rent				$	200
Insurance				$	8
Other Overhead (Professional Fees, Education & Travel, etc)				$	10
Total Expenses				$	11,588
PROFIT				$	1,154

Michigan Eggplant
WITHOUT Methyl Bromide

	Quantity	Unit	Price per Unit		Total per Acre
Revenue					
Eggplant	650	1 1/9 bu boxes	$ 7.74	$	5,034
Total Revenue				$	5,034
Expenses					
Field Preparation					
Cover Crop (Materials, Machinery & Labor)				$	13
Field tillage				$	39
Soil Test				$	2
Lime & Application				$	13
Fertilizer (Materials)				$	100
Apply & Incorporate Fertilizer (Machinery & Labor)				$	34
Plastic & Drip Tape (Materials)				$	284
Lay Plastic & Drip Tape (Machinery & Labor)				$	98
Fumigate (Materials)					
Herbicide, Insecticide & Fungicide (Materials)				$	231
Apply Herbicide, Insecticide & Fungicide (Mach & Lab)				$	90
Plant & Grow					
Transplants				$	848
Planting				$	75
Staking & Tieing (Machinery, Labor & Materials)				$	258
Cultivate & Hoe				$	129
Drip Irrigate & Fertigate (Labor, Materials & Electricity)				$	267
Scouting & Lab Work				$	30
Field Maintenance - Driveways & Mowing (Mach & Lab)				$	7
Harvest					
Pick Crop (Field to Packing Shed)				$	780
Grading & Packing (Includes shipping containers)				$	1,944
Shipping (Includes Materials, Machinery & Labor)				$	325
Sales & Marketing	9%	of gross		$	420
Field Clean-up				$	112
Management & Supervision				$	75
Interest on Operating Capital		8%		$	101
Land Rent				$	200
Insurance				$	8
Other Overhead (Professional Fees, Education & Travel, etc)				$	10
Total Expenses				$	6,492
PROFIT				$	(1,458)

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ID# ___

Worksheet 3-A(1). Alternatives - Technical Feasibility of Alternatives to Methyl B........

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative. Use additional pages as need.
For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).
When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.
Summarize each of the research studies you cite in the Research Summary Worksheet.
If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work:
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: 1,3-Dichloropropene, Chloropicrin **Study:** UNEP 1998, B-83, B-281, B-87, B-287

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted ___X___
1b. Township caps ______
1c. Alternative not acceptable in consuming country ______
1d. Other (Please describe) ______

______
______
______
______

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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ID# ______________

Worksheet 3-A(1). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes X No ____

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ____________________

3. Publication and Date of Publication ____________________

4. Location of research study ____________________

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

____

6. Was crop yield measured in the study? Yes ____ No ____

7. Describe the effectiveness of the alternative in controlling pests in the study.

____

8. Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?

These studies listed in UNEP 1998 do not indicate that these treatments are effective against *P. capsici.*
In contrast, many studies indicate that the alternatives of chloropicrin and 1,3-dichloropropene are not effective.

Worksheet 3-A(1)(b). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. <u>You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative</u> Use additional pages as need.

<u>For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed</u>. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: <u>1,3-Dichloropropene, Chloropicrin</u> Study: <u>UNEP 1998, UNEP 2000, B-5, B-37, B-51, B-54, D-107</u>

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted ____X____
1b. Township caps ________
1c. Alternative not acceptable in consuming country ________
1d. Other (Please describe) ________

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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ID# ____________

Worksheet 3-A(1)(b). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes X No ____

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ____________

3. Publication and Date of Publication ____________

4. Location of research study ____________

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

   1,3-D, chloropicrin

6. Was crop yield measured in the study? Yes ____ No ____

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?

   These studies do not indicate that these treatments are effective against *P. capsici*. In contrast, many studies indicate that the alternatives of chloropicrin and 1,3-dichloropropene are not effective.

Worksheet 3-A(2). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous p not effective for your conditions. This worksheet contains 9 questions. <u>You must complete one copy of workst each research study you use to evaluate a single methyl bromide alternative</u> Use additional pages as need.

<u>For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed</u>. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: <u>1,3-Dichloropropene, Chloropicrin, Pebulate</u> Study: <u>UNEP 1998, B-51, B-72</u>

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted ___X___
1b. Township caps ______
1c. Alternative not acceptable in consuming country ______
1d. Other (Please describe) ______

____
____
____
____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

For EPA Use Only
ID# ________________

Worksheet 3-A(2). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes X No ____

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ________________

3. Publication and Date of Publication ________________

4. Location of research study ________________

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

   1,3-D, chloropicrin, Pebulate

6. Was crop yield measured in the study? Yes ____ No ____

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?

   These studies do not indicate that these treatments are effective against *P. capsici*. In contrast, many studies indicate that the alternatives of chloropicrin and 1,3-dichloropropene are not effective. Pebulate is used for nematode and weed control and is not effective against *P. capsici*.

OMB Control # 2060-0482

For EPA Use Only
ID#__________

Worksheet 3-A(3). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. <u>You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative</u>. Use additional pages as need.

<u>For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed</u>. number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research s the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: 1,3-D, Metam Sodium, Basamid   Study: UNEP 1998, B-281

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted __X__
1b. Township caps _____
1c. Alternative not acceptable in consuming country _____
1d. Other (Please describe) _____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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Worksheet 3-A(3). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes __X__ No ______

   1a. *If not on the EPA website, please attach a copy.*

2. Author(s) or researcher(s) ______________

3. Publication and Date of Publication ______________

4. Location of research study ______________

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

   1,3-D, Metam Sodium, Basamid

6. Was crop yield measured in the study? Yes ______ No ______

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. *Would you expect similar results?* Are there other factors that would affect your adoption of this tool?

   The study in UNEP 1998 B-281 indicates that direct injection of metam sodium in bands to soil does not provide consistent control due to non-uniform distribution in the soil. Also, problems with microorganisms that degrade the chemical, thereby making it less effective, have been noted. Basamid is a product primarily used to manage nematodes and weeds, and not for disease control.

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ID# ____________

Worksheet 3-A(4). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative Use additional pages as need.

For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Basamid **Study:** UNEP 1998, B-87, B-284, B-287

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted ___X___
1b. Township caps ______
1c. Alternative not acceptable in consuming country ______
1d. Other (Please describe) ______

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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ID# ____________

Worksheet 3-A(4). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes X No ____

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ____________

3. Publication and Date of Publication ____________

4. Location of research study ____________

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

   Basamid

6. Was crop yield measured in the study? Yes ____ No ____

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?

   Basamid is a product primarily used to manage nematodes and weeds, and not for disease control.

For EPA Use Only
ID#____________

Worksheet 3-A(5). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative. Use additional pages as need.

For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Resea Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Basamid, Soil Solarization **Study: UNEP 1998, B-49**

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted ___X___
1b. Township caps ________
1c. Alternative not acceptable in consuming country ________
1d. Other (Please describe) ________

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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ID# ____________

Worksheet 3-A(5). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes X No ____

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ____________

3. Publication and Date of Publication ____________

4. Location of research study ____________

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

   Basamid, Soil Solarization

6. Was crop yield measured in the study? Yes ____ No ____

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?

   Basamid is a product primarily used to manage nematodes and weeds, and not for disease control. Solarization of the soil to kill the overwintering spores is not feasible in a northern state where the growing season is hort (May to September), and cold temperatures (<50 F) prevail through much of the year. Also, this management strategy has not been proven effective for *Phytophthora capsici*.

OMB Control # 2060-0482

Worksheet 3-A(6). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. <u>You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative</u> Use additional pages as need.

<u>For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed</u>. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) cou... successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Metam Sodium **Study:** UNEP 1998, B-52, B-83, B-87, B-281, B-287

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted ___X___
1b. Township caps ________
1c. Alternative not acceptable in consuming country ________
1d. Other (Please describe) ________

____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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Worksheet 3-A(6). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. **Is the study on EPA's website?** Yes X No ____

   1a. **If not on the EPA website, please attach a copy.**

2. **Author(s) or researcher(s)** ____________

3. **Publication and Date of Publication** ____________

4. **Location of research study** ____________

5. **Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.**

   Metam Sodium

6. **Was crop yield measured in the study?** Yes ____ No ____

7. **Describe the effectiveness of the alternative in controlling pests in the study.**

8. **Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?**

   Metam sodium is not considered a good product against fungi, especially *Phytophthora* , but rather is used for weed control and for nematode problems. Also, problems with microorganisms that degrade the chemical, thereby making it less effective, have been noted.

Worksheet 3-A(6)(b). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. <u>You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative</u> Use additional pages as need.

<u>For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed</u>. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Metam Sodium **Study: Alternatives for methyl bromide on cucurbits and solanaceous crops, 2002.**

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted X
1b. Township caps ____
1c. Alternative not acceptable in consuming country ____
1d. Other (Please describe) ____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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Worksheet 3-A(6)(b). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. **Is the study on EPA's website?** **Yes** ____ **No** X

   1a. If not on the EPA website, please attach a copy.

2. **Author(s) or researcher(s)** M.K. Hausbeck
   B.D. Cortright

3. **Publication and Date of Publication** Research in progress

4. **Location of research study** Michigan, USA

5. **Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.**
   Multigard FFA, Multigard Protect, Multigard Protect + Vapam HL, CX-100

6. **Was crop yield measured in the study?** **Yes** ____ **No** X

7. **Describe the effectiveness of the alternative in controlling pests in the study.**
   Fields have not been harvested yet.

8. **Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?**

   The results of this study are directly applicable, since the research was conducted in Michigan, USA.

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Worksheet 3-A(7). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative. Use additional pages as need.

For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list c alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Metam Sodium, Crop Rotation **Study:** UNEP 1998, B-39, B-74

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted X
1b. Township caps ____
1c. Alternative not acceptable in consuming country ____
1d. Other (Please describe) ____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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Worksheet 3-A(7). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. **Is the study on EPA's website?** **Yes** X **No** ______

   1a. **If not on the EPA website, please attach a copy.**

2. **Author(s) or researcher(s)** ____________

3. **Publication and Date of Publication** ____________

4. **Location of research study** ____________

5. **Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.**

   Metam Sodium, Crop Rotation

6. **Was crop yield measured in the study?** **Yes** ______ **No** ______

7. **Describe the effectiveness of the alternative in controlling pests in the study.**

8. **Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?**

   The efficacy of crop rotation depends on the life cycle of the pathogen and its ability to overwinter and persist in soils. *Phytophthora capsici* has an overwintering structure called an oospore that is capable of surviving for long periods of time, thereby negating the benefits of crop rotation. Metam sodium is not considered a good product against fungi, especially *Phytophthora*, but rather is used for weed control and for nematode problems. The rotation suggested in this study primarily lists crops that are susceptible to *P. capsici*, including pepper, cucumber, tomato and squash. Using this suggested rotation would exacerbate the disease problem. Therefore, this alternative would not be effective for Michigan growers.

Worksheet 3-A(8). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative Use additional pages as need.

For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Methyl Iodide **Study: UNEP 2001, E-72**

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted ____
1b. Township caps ____
1c. Alternative not acceptable in consuming country X
1d. Other (Please describe) ____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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Worksheet 3-A(8). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. **Is the study on EPA's website?** **Yes** X **No** ____

   1a. If not on the EPA website, please attach a copy.

2. **Author(s) or researcher(s)** ________________

3. **Publication and Date of Publication** ________________

4. **Location of research study** ________________

5. **Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.**

   Methyl Iodide

6. **Was crop yield measured in the study?** **Yes** ____ **No** ____

7. **Describe the effectiveness of the alternative in controlling pests in the study.**

   ____

8. **Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?**

   Methyl iodide is not registered in the U.S., and has not been proven to be effective against the soil-borne organism *Phytophthora*.

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Worksheet 3-A(9). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. <u>You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative</u>. Use additional pages as need.

<u>For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed</u>. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as a application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, r outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Propargyl bromide **Study:** UNEP 2001, E-73

Section I. Initial Screening on Technical Feasibility of Alternatives

1. **Are there any location-specific restrictions that inhibit the use of this alternative on your site?**

1a. Full use permitted ______
1b. Township caps ______
1c. Alternative not acceptable in consuming country __X__
1d. Other (Please describe) ______

____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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Worksheet 3-A(9). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes X No ____

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ______________

3. Publication and Date of Publication ______________

4. Location of research study ______________

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

   Propargyl Bromide

6. Was crop yield measured in the study? Yes ____ No ____

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?

   Propargyl bromide is not registered in the U.S., and has not been proven to be effective against the soil-borne organism *Phytophthora*.

Worksheet 3-A(10). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative Use additional pages as need.

For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website a websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and theref... research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Biofumigation **Study:** UNEP 1995, UNEP 1998, A-71, B-41, B-83, B-87, B-91 B-92, B-94

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted ___X___
1b. Township caps ______
1c. Alternative not acceptable in consuming country ______
1d. Other (Please describe) ______

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

Worksheet 3-A(10). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes X No ____

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ______________________

3. Publication and Date of Publication ______________________

4. Location of research study ______________________

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

   Biofumigation

6. Was crop yield measured in the study? Yes ____ No ____

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?

   Biofumigation does not readily apply to Michigan's situation. The reasons for this include the lack of evidence that this treatment works for *Phytophthora capsici* under Michigan's cool climate. *Phytophthora*'s oospore would not be killed using biofumigation in Michigan's soils. Beneficial predatory nematodes important for biofumigation have not been identified or quanitified in Michigan's vegetable growing regions.

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Worksheet 3-A(10)(b). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. <u>You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative</u> Use additional pages as need.

<u>For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed</u>. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Biofumigation **Study: Alternatives for methyl bromide on cucurbits and solanaceous crops, 2002.**

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted __X__
1b. Township caps _____
1c. Alternative not acceptable in consuming country _____
1d. Other (Please describe) _____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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Worksheet 3-A(10)(b). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. **Is the study on EPA's website?** **Yes** ______ **No** X

   1a. **If not on the EPA website, please attach a copy.**

2. **Author(s) or researcher(s)** M.K. Hausbeck
   B.D. Cortright

3. **Publication and Date of Publication** Research in progress

4. **Location of research study** Michigan, USA

5. **Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.**
   Multigard FFA, Multigard Protect, Multigard Protect + Vapam HL, CX-100

6. **Was crop yield measured in the study?** **Yes** ______ **No** X

7. **Describe the effectiveness of the alternative in controlling pests in the study.**
   Fields have not been harvested yet.

8. **Discuss how the results of the study apply to your situation. Would you expect similar results? Are there *other factors that would affect your adoption of this tool?***

   The results of this study are directly applicable, since the research was conducted in Michigan, USA.

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Worksheet 3-A(11). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative. Use additional pages as need.

For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternative the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Solarization **Study:** UNEP 1995, UNEP 1998, A-100, B-49, B-71, B-83, B-92, B-94, B-281, A-77, B-74, B-86, B-87, B-286, B-287

Section I. Initial Screening on Technical Feasibility of Alternatives

1. **Are there any location-specific restrictions that inhibit the use of this alternative on your site?**

   1a. Full use permitted X
   1b. Township caps ______
   1c. Alternative not acceptable in consuming country ______
   1d. Other (Please describe) ______

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

For EPA Use Only
ID# ______________

Worksheet 3-A(11). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes X No ____

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ______________

3. Publication and Date of Publication ______________

4. Location of research study ______________

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

   Solarization

6. Was crop yield measured in the study? Yes ____ No ____

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?

   Solarization of the soil to kill the overwintering spores is not feasible in a northern state where the growing season is short (May to September), and cold temperatures (<50 F) prevail through much of the year. Also, this management strategy has not been proven effective for *Phytophthora capsici*.

For EPA Use Only
ID# ______________

Worksheet 3-A(12). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative. Use additional pages as need.

For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
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(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Solarization, Fungicides **Study:** UNEP 1998, B-71

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted X
1b. Township caps ______
1c. Alternative not acceptable in consuming country ______
1d. Other (Please describe) ______

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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ID# ______________

Worksheet 3-A(12). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes X No ____

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ____________

3. Publication and Date of Publication ____________

4. *Location of research study* ____________

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

   Solarization, Fungicides

6. Was crop yield measured in the study? Yes ____ No ____

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?

   Solarization of the soil to kill the overwintering spores is not feasible in a northern state where the growing season is short (May to September), and cold temperatures (<50 F) prevail through much of the year. Also, this management strategy has not been proven effective for *Phytophthora capsici*. Resistance has developed to registered fungicides.

For EPA Use Only
ID#_______________

Worksheet 3-A(12)(b). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative. Use additional pages as need.

For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Fungicides

Study: The dynamics of mefenoxam insensitivity in a recombining population of *Phytophthora capsici* characterized with amplified fragment length polymorphism markers.

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted ___X___
1b. Township caps ______
1c. Alternative not acceptable in consuming country ______
1d. Other (Please describe) ______

____

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If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

For EPA Use Only
ID# ______________

Worksheet 3-A(12)(b). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. **Is the study on EPA's website?** **Yes** ______ **No** X

   1a. If not on the EPA website, please attach a copy.

2. **Author(s) or researcher(s)** K.H. Lamour
   M.K. Hausbeck

3. **Publication and Date of Publication** Phytopathology 91:553-557, 2002

4. **Location of research study** Michigan, USA

5. **Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.**
   Mefenoxam

6. **Was crop yield measured in the study?** **Yes** ______ **No** X

7. **Describe the effectiveness of the alternative in controlling pests in the study.**
   Insensitivity of *Phytophthora capsici* to mefenoxam, a commonly used fungicide, is common in Michigan fields. Insensitivity of the pathogen to this fungicide renders this treatment ineffective.

8. **Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?**

   The results of this study are directly applicable since the research was conducted in Michigan, USA.

The Dynamics of Mefenoxam Insensitivity in a Recombining Population of *Phytophthora capsici* Characterized with Amplified Fragment Length Polymorphism Markers

K. H. Lamour and M. K. Hausbeck

Department of Botany and Plant Pathology, Michigan State University, East Lansing 48824.
Accepted for publication 12 February 2001.

ABSTRACT

Lamour, K. H., and Hausbeck, M. K. 2001. The dynamics of mefenoxam insensitivity in a recombining population of *Phytophthora capsici* characterized with amplified fragment length polymorphism markers. Phytopathology 91:553-557.

Recent findings from Michigan suggest that recombination may play a role in the survival and evolution of sensitivity to the fungicide mefenoxam in populations of *Phytophthora capsici* on cucurbit hosts. In 1998, 63 mefenoxam insensitive isolates were recovered from a squash field in which mefenoxam had been applied. Additional isolates were recovered from untreated squash fields planted at this location in 1999 (200 isolates) and the spring of 2000 (34 isolates). Isolates from 1998 and 1999 were characterized using fluorescent amplified fragment length polymorphism (AFLP) markers and all isolates were screened for compatibility type and mefenoxam sensitivity. In 1998 and 1999, 92 and 71% of the isolates, respectively, had unique multilocus AFLP genotypes with no identical isolates recovered between years. Seventy-two identical AFLP markers were clearly resolved in both the 1998 and 1999 sample sets, and fixation indices for the 37 polymorphic AFLP loci indicate little differentiation between years. There was no decrease in the frequency of resistant isolates during the 2 years without mefenoxam selection. We conclude that oospores play a key role in overwintering and that the frequency of mefenoxam insensitivity may not decrease in an agriculturally significant time period (2 years) once mefenoxam selection pressure is removed.

Additional keywords: fungicide resistance, genetic diversity, population genetics.

Crown, root, and fruit rot caused by *Phytophthora capsici* is increasing in Michigan cucurbit production fields, and uninfested land suitable for rotation is becoming increasingly scarce, especially in areas undergoing rapid urban development. The phenylamide fungicide (PAF) mefenoxam is a systemic fungicide that appears to be acting at the level of DNA translation, and is fungistatic to fully sensitive isolates of *P. capsici* (2,13). Although mefenoxam has been considered by some growers to be helpful, mefenoxam insensitive isolates were reported on bell peppers in North Carolina and New Jersey by Parra and Ristaino in 1998 (18) and have since been recovered from 10 of 11 farms sampled in Michigan (13), as well as, in Georgia (15) and southern Italy (19). Mefenoxam insensitivity in Michigan *P. capsici* isolates is inherited as a single gene exhibiting incomplete dominance (13), which is consistent with the reports for a variety of other oomycetous organisms (2). Investigations with *P. infestans* indicate that insensitivity may be conferred by genes at different chromosomal positions (5), suggesting that the basis of insensitivity in different populations may not be identical. Sexual recombination, in particular, has the potential to impact management strategies that employ PAFs because the fully insensitive (two copies of the insensitivity allele) phenotype may be directly generated. *P. capsici* is heterothallic and the sexual stage is initiated when isolates of opposite compatibility type, designated A1 and A2, come into close association to form thick-walled oospores (4). The asexual stage includes the production of caducous sporangia born on long pedicels, which may release motile zoospores if free water is present. Asexual spores are thought to be responsible for the polycyclic nature of disease development (20).

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Publication no. P-2001-0312-03R

PAF resistance in the genus *Phytophthora* and, in particular, the *P. infestans*–potato pathosystem, is well documented (2,4,9). Until recently, the population structure of *P. infestans* appeared to be largely clonal outside of *P. infestans* putative center of origin (6). The recent detection of both *P. infestans* compatibility types along with increased genotypic diversity in some potato growing regions indicates that the sexual stage is likely active and may significantly impact control strategies that have proved useful in the past (3,8). When PAF resistance in European *P. infestans* populations increased significantly in the early 1980s, the efficacy of the PAF metalaxyl was only regained after the product was not made available to growers for a period of time (2). This strategy apparently allowed the resistant populations to decline or become extinct and depends on ephemeral populations or, in the case of resident populations, upon a significant cost for resistance outside of selection pressure. A recent study of sensitive versus PAF resistant *P. nicotianae* isolates from citrus suggests negligible fitness costs for PAF resistance and reports that 2 years without PAF use did not reduce the proportion of resistant isolates in groves (21). Kadish and Cohen report that PAF-resistant *P. infestans* isolates in Israel were more aggressive in colonizing tuber tissue than sensitive isolates (12).

Novel techniques have been developed recently that allow characterization of DNA-level polymorphism in organisms for which little is known about the genome. An example is the amplified fragment length polymorphism (AFLP) technique introduced by Vos et al. in 1995 (23). This technique relies on restriction enzyme fragmentation of genomic DNA with the concomitant ligation of synthetic adaptors to the DNA fragment ends. Stringent polymerase chain reaction (PCR) amplification using adaptor-complementary primers with additional selective nucleotides allow for the amplification of fragment subsets. DNA fragment subsets are termed fingerprints and may be resolved with a range of techniques (1). AFLP markers have been used on a variety of organisms (14,22) and the procedure generates a large number of reproducible markers (1,22). The limitation that these markers are generally scored as dominant markers (e.g., either present or absent) for diploid organisms requires the use of relatively large sample sets (11,25).

Our null hypotheses are that sexual recombination has a significant impact on the population structure of *P. capsici* in Michigan and that mefenoxam insensitivity may not decrease in the time frame of a typical 2-year rotation outside of mefenoxam selection pressure.

MATERIALS AND METHODS

Field plot. Research was conducted on a commercial farm in southwest Michigan, with a history (>11 years) of *P. capsici* on bell peppers and squash and intensive use of PAF. The 4.05-ha field sampled had previously been cropped to soybeans and corn with no known record of *P. capsici* susceptible crops (e.g., tomatoes, peppers, or cucurbits) prior to 1997. During 1997 and 1998, yellow squash and zucchini grown in this field became diseased with *Phytophthora* crown, root, and fruit rot and the grower applied mefenoxam as part of a disease management strategy (Novartis, Greensboro, NC). In 1998, all isolates recovered were either intermediately or fully insensitive to mefenoxam. Both A1 and A2 compatibility types were present, and oospores were detected in diseased fruit. In 1999 and 2000, yellow squash was established in a 1,124-m^2 experimental plot in this field, and mefenoxam was not applied. Diseased plants and fruit were sampled on 20 August 1998 (63 isolates from entire field), June through August 1999 (200 isolates from experimental plot), and 13 July 2000 (34 isolates from experimental plot). All isolates were recovered from single diseased plants or fruit.

TABLE 1. Fixation indices (F_{ST}) for 37 amplified fragment length polymorphism loci from unique *Phytophthora capsici* isolates collected from a single Michigan cucurbit field during 1998 ($N = 57$) and 1999 ($N = 141$)

Fragment[a]	1998 f(aa)[b]	1999 f(aa)	F_{ST}[c]
45	0.02	0.06	0.018
54	0.29	0.29	0.000
64	0.82	0.55	0.048
104	0.11	0.06	0.007
106	0.11	0.04	0.025
110	0.41	0.36	0.002
130	0.41	0.30	0.009
146	0.47	0.24	0.038
149	0.12	0.27	0.029
154	0.39	0.31	0.004
156	0.53	0.83	0.054
172	0.56	0.33	0.034
189	0.16	0.56	0.121
192	0.16	0.37	0.044
193	0.35	0.20	0.022
211	0.47	0.15	0.088
241	0.48	0.32	0.018
256	0.04	0.01	0.022
258	0.43	0.49	0.002
261	0.55	0.54	0.000
270	0.57	0.41	0.015
282	0.35	0.40	0.002
285	0.51	0.73	0.030
314	0.51	0.34	0.019
320	0.41	0.51	0.006
333	0.16	0.20	0.002
346	0.36	0.33	0.001
361	0.33	0.49	0.017
383	0.21	0.15	0.005
418	0.40	0.34	0.002
431	0.34	0.32	0.001
438	0.67	0.45	0.028
454	0.65	0.49	0.015
492	0.29	0.40	0.009
504	0.51	0.47	0.001
511	0.38	0.28	0.007
548	0.78	0.78	0.000

[a] *Eco*RI-AC/*Mse*I-CA selectively amplified fragment size in base pairs.

[b] Observed frequency of the absent state where "a" represents the absence of a fragment.

[c] F_{ST} calculated from estimated allele frequencies. According to Wright's qualitative guidelines, values from 0 to 0.05 indicate little genetic differentiation and values from 0.05 to 0.15 indicate moderate genetic differentiation.

Isolate collection and maintenance. Isolation from diseased plant material was made onto BARP (25 ppm of benomyl, 100 ppm of ampicillin, 30 ppm of rifampicin, and 100 ppm of pentachloronitrobenzene)-amended UCV8 (840 ml of distilled water, 163 ml of unclarified V8 juice, 3 g of $CaCO_3$, and 16 g of Bacto agar) plates. Procedures for obtaining single zoospore isolates were as previously described (13). Single zoospore cultures were maintained on 30 ppm of rifampicin and 100 ppm of ampicillin (RA)-UCV8 plates and transferred bimonthly. Long-term storage consisted of a single 7-mm plug of expanding mycelium from each single zoospore culture being placed in a 1.5-ml microfuge tube with one sterilized hemp seed and 1 ml of sterile distilled water, incubated for 2 to 3 weeks at 23 to 25°C, and stored at 15°C long term.

Phenotypic characterization. Isolates were screened for compatibility type as previously described (13). Mefenoxam sensitivity was characterized according to the in vitro screening technique described by Lamour and Hausbeck (LH technique) for *P. capsici* isolates in Michigan (13). Isolates were scored as sensitive (S) if growth on UC-V8 agar amended with 100 ppm of mefenoxam was less than 30% compared with a control, as intermediately sensitive (IS) if between 30 and 90%, and fully insensitive (I) if greater than 90% compared with the unamended control. These mefenoxam sensitivity categories are based on a trimodal distribution of 523 field isolates of *P. capsici*. Clear modal distributions were only attained when screening was conducted with a single high rate of mefenoxam-amended (100 ppm) media (K. Lamour, *unpublished data*). These putative mefenoxam sensitivity categories were tested by in vitro crosses (I × S, IS × IS, IS × S, and S × S), and chi-square analysis confirmed that the observed progeny numbers were not significantly different than expected for Mendelian inheritance of an incompletely dominant trait (13).

The LH technique differs from a commonly used method described by Goodwin, Sujkowski, and Fry (GSF technique) (9) for *P. infestans* which uses two levels of amended media (5 and 100 ppm) to differentiate the three mefenoxam sensitivity phenotypes and which has been used to characterize *P. capsici* isolates (15,18,19). Unfortunately, analysis of our in vitro crosses and field isolates by the GSF technique did not resolve a clear modal distribution (K. Lamour, *unpublished data*). Assignment of Michigan *P. capsici* isolates to the S category was the same whether using the LH or GSF technique. The only difference was that some *P. capsici* isolates from Michigan rated as fully insensitive by the GSF technique were rated as intermediately sensitive by the LH technique.

DNA extraction and AFLP fingerprinting. A technique for avoiding bacterial contamination prior to growing isolates for DNA extraction was implemented using a modified Van Teigham cell (4). The uppermost portion of a 7-mm plug of mycelium was placed onto the surface of RA-WA plates (30 ppm of rifampicin, 100 ppm of ampicillin, 1,000 ml of distilled water, and 16 g of Bacto agar) and an autoclaved cap from a 1.5-ml microfuge tube was placed over the plug which forced the isolate to grow through the amended media. Isolates were incubated in the dark for 2 t 3 days before two 7-mm plugs were transferred to approximately 15 ml of RA-UCV8 broth in petri dishes (100 × 15 mm) and incubated in the dark for 3 days at 23 to 25°C. Mycelial mats were washed with distilled water and dried briefly under vacuum before being frozen to –20°C and lyophilized.

Lyophilized mats were ground with a sterile mortar and pestle. Whole genomic DNA from approximately 50 mg of ground mycelium was extracted with a plant mini kit (Dneasy; Qiagen Inc., Valencia, CA) according to the manufacturers directions. DNA was quantified (Nucleic Acid QuickSticks; Clontech, Palo Alto, CA) according to the manufacturers directions and approximately 100 ng of DNA was subjected to a restriction/ligation reaction, preselective amplification, and selective amplifications using the PCR core mix, adaptor sequences, core primer sequences, and fluorescent-labeled primers available in an AFLP microbial fingerprinting kit (Perkin-Elmer Applied Biosystems, Foster City, CA, henceforth referred to as PE/ABI) and performed exactly as described in protocol part 402977 Rev A (23). All PCR reactions were performed with a minicycler (MJ Research Inc., Waltham, MA) in 0.2-ml tubes according to the cycling parameters outlined in the microbial fingerprinting protocol.

An initial optimization set of reactions was performed with preselective products from *P. capsici* isolate OP97, which was isolated from a cucumber fruit in 1997 (13). Selective amplifications with the selective primers *Eco*RI-AA, -AC, -AG, and -AT were performed in all 16 combinations with the *Mse*I-CA, -CC, -CG, and -CT selective primers. *Eco*RI selective primers, available from PE/ABI, were labeled at the 5′ end with either carboxyfluorescein (FAM), carboxytetramethyrhodamine (TAMRA), or carboxy-4′,5′-dichloro-2′,7′-dimethoxyfluorescein (JOE) fluorescent dyes. The fluorescent dyes are excited by laser radiation and visualized by their characteristic absorption-emission frequencies. Only the fragments containing an *Eco*RI restriction site are resolved.

Products from three reactions labeled with different colored dyes and a carboxy-X-rhodamine (ROX) size standard were loaded into each lane on a denaturing polyacrylamide gel and the fragments resolved in a DNA sequencer (ABI Prism 377). Results were prepared for analysis in the form of electropherograms using GeneScan Analysis software (PE/ABI). AFLP fragments were scored manually as present (1) or absent (0) using Genotyper (PE/ABI). Only DNA bands that consistently exhibited unambiguous presence or absence profiles were scored.

A single isolate, OP97, was subjected to the aforementioned protocol using three primer pair combinations that were chosen as optimal on three separate occasions, approximately 3 months apart, to test for reproducibility of AFLP profiles.

Clone detection and cluster analysis. AFLP fragments were considered polymorphic if the most common allele was present in less than 95% of the isolates from a given sample set and scored for presence (1) or absence (0) (10). AFLP fragments present in more than 95% of the isolates from a given sample set were considered monomorphic. Analysis of the resulting binary data matrix was performed using NTSYS-pc version 2.02k (Exeter Software, Setauket, NY). Unweighted pair group method with arithmetic averages cluster analysis was performed on the matrix of similarity coefficients calculated from all possible pairwise comparisons of individuals within and among the 1998 and 1999 populations and a tree generated. Isolates showing complete homology at all loci were considered to be clones and except for a single representative isolate were excluded from frequency calculations.

Allele frequency and fixation indices. Allele frequencies for AFLP markers were estimated utilizing the expected relationship between gene and genotype frequencies in a randomly mating population (i.e., Hardy-Weinberg proportions). The frequency of the recessive (absent) allele (q) was calculated from the observed number of recessive homozygote individuals (X) in a sample of n individuals by the formula for dominant markers described by Jorde et al. (11):

$$\hat{q} = \sqrt{x + \frac{1-x}{4n}}$$

where $x = X/n$ is the observed proportion of individuals that do not display the dominant (present) marker phenotype. In order to test whether the composite genetic profiles from 1998 and 1999 were consistent with a single randomly mating population, the fixation index was calculated for each AFLP loci from the variance in allele frequencies according to the following formula: $F_{ST} = [(p_1 - p_2)^2/4]/(\text{average } p \times \text{average } q)$, where p is the allele frequency for the present state with p_1 and p_2 indicating the two sample populations, and q is the allele frequency for the absent state (10). Fixation indices for individual loci were interpreted according to the qualitative guidelines suggested by Wright (24), where the range 0 to 0.05 indicates little genetic differentiation, range 0.05 to 0.15 indicates moderate genetic differentiation, and greater than 0.25 indicates great genetic differentiation (10).

RESULTS

AFLP band characterization. Evaluation of the 16 *Eco*RI + 2–*Mse*I + 2 selective primer pair combinations indicated that *Eco*RI + AC–*Mse*I + CA gave the most clearly resolved fragment profile and was used to amplify genomic DNA from all isolates in both the 1998 and 1999 sample sets. This primer combination resulted in 72 clearly resolved fragments of which 37 (51%) fragments were polymorphic in both 1998 and 1999 (Table 1). All 72 fragments were present in both 1998 and 1999 and no novel fragments were detected between years. The following 35 fragments (size in base pairs) were monomorphic in both the 1998 and 1999 sample sets: 41, 43, 47, 49, 58, 66, 70, 82, 85, 114, 118, 123, 133, 135, 140, 159, 174, 235, 247, 249, 272, 278, 295, 298, 300, 341, 351, 355, 367, 402, 474, 488, 502, 519, and 527. AFLP profiles for isolate OP97, generated from separate DNA extractions on three separate occasions over a 1-year period, resulted in identical banding patterns with the only difference being minor changes in the intensity of the electropherogram signal. Occasionally individual reactions resulted in poorly resolved fingerprint profiles (e.g., low intensity of signal) and were repeated until signals were deemed optimal.

Phenotypic, genotypic, and gene diversity. No isolates sensitive to mefenoxam were recovered in 1998 or 2000, and single A1 sensitive and A2 sensitive isolates were recovered in 1999 (Table 2). In 1998, 18% of the isolates were intermediately sensitive and 82% were insensitive, in 1999, 2% were sensitive, 28% were intermediately sensitive and 70% were insensitive, and in 2000, 15% of the isolates were intermediately sensitive and 85% were insensitive to mefenoxam (Table 2).

In 1998, 57 of the 63 isolates recovered, and 141 of the 200 isolates recovered in 1999 were unique based on multilocus AFLP profiles. No identical multilocus genotypes were recovered between 1998 and 1999. Five isolates (two A2/I, two A2/IS, and

TABLE 2. Phenotypic diversity of *Phytophthora capsici* isolates recovered from the same cucurbit field in 1998, 1999, and 2000

Year[a]	No. of isolates[b]	Compatibility type and mefenoxam sensitivity[c]					
		A1/S	A1/IS	A1/I	A2/S	A2/IS	A2/I
1998	57	–	4	31	–	6	16
1999	141	1 (2)	17 (20)	57 (53)	1 (1)	23 (18)	42 (47)
2000	34	–	2	8	–	3	21

[a] Mefenoxam was applied in 1998 but not in 1999 or 2000.

[b] Sample sets from 1998 and 1999 consist of unique multilocus genotypes as determined with amplified fragment length polymorphism fingerprinting. The 2000 sample set was recovered at the beginning of the growing season and was not fingerprinted.

[c] S = sensitive, IS = intermediately sensitive, and I = insensitive as determined by in vitro screening on 100 ppm of mefenoxam-amended agar. Numbers in parentheses indicate the expected number of isolates when mefenoxam insensitivity is assumed to be controlled by a single incompletely dominant gene in Hardy-Weinberg equilibrium unlinked to compatibility type.

one A1/I) of *P. capsici* collected in 1998 had one clonal representative. Fourteen isolates collected in 1999 had between two and four clones (Table 3). A single A1 compatibility type insensitive isolate had 40 clones recovered over the course of the 1999 season and comprised 3% of the early, 15% of the mid-, and 43% of the late sampling intervals (Table 3). The 1999 sampling intervals (early, mid, and late) are based on the dates of sampling and are not intended to reflect stages of plant growth or the epidemiology of *P. capsici*. Cluster analysis of AFLP fingerprint variation indicated no significant clustering of isolates between 1998 and 1999.

The majority (98%) of the 37 polymorphic AFLP fragments showed little genetic differentiation ($F_{ST} < 0.05$) between 1998 and 1999 according to Wrights qualitative criterion (Table 1) (24).

DISCUSSION

P. capsici causes significant damage to cucurbit hosts in Michigan each year. In an effort to prevent or control epidemics, many growers have used either metalaxyl or the newer, but similarly acting compound, mefenoxam as a part of their disease management strategy. This study was initiated in an effort to address the concerns of growers who have high levels of mefenoxam insensitivity.

Phenotypic data (mefenoxam sensitivity and compatibility type) from a 1998 survey suggested that insensitivity to mefenoxam was common and that some level of recombination is occurring in the field (13), but without the application of additional polymorphic markers our ability to assess population structure was severely restricted. AFLP analysis proved to be a powerful tool for resolving the population dynamics of *P. capsici*. A single selective primer combination, *Eco*RI-AC–*Mse*I-CA, generated 72 bands of which 37 were polymorphic in our 1998 and 1999 sample sets. AFLP fingerprinting, in conjunction with temporal sampling, provided a useful characterization of *P. capsici* from one season to the next and allowed us to track asexual disease development over the course of a single season.

Our data suggests that sexual recombination significantly impacts the structure of this *P. capsici* population. The finding that 198 of the 262 isolates recovered between 1998 and 1999 had unique multilocus AFLP genotypes is consistent with the high level of genotypic diversity expected in an outcrossing population (7,16,17). Although clonal reproduction occurred in 1998 and 1999, no identical genotypes were recovered between years, suggesting that oospores are important for overwintering. The finding that 35 of the 37 polymorphic fragments exhibited very little differentiation (i.e., change in allele frequency) based on the estimated fixation indices between 1998 and 1999 is consistent with the expectations for a recombining population large enough to avoid dramatic changes due to genetic drift.

In 1999 and 2000, sensitive and intermediately sensitive isolates (42 of 175) did not increase in a manner suggesting selection in favor of mefenoxam sensitivity outside of mefenoxam selection pressure. The fact that 14 of the 15 isolates with clonal reproduction in 1999 were fully insensitive may be another indication that mefenoxam insensitivity does not have significant costs outside of mefenoxam selection pressure. If we assume that there is only a single mefenoxam insensitivity gene in this population unlinked to compatibility type, designated *I*, and that this population is effectively free from the effects of migration and genetic drift, some interesting speculations can be made. For instance, in 1999, if the mefenoxam sensitivity phenotypes are assumed to represent genotypes (e.g., a fully insensitive isolate has two copies of the *I* allele) then the frequency of *I* can be estimated and the observed number of unique isolates that fall into each of the six mefenoxam sensitivity/compatibility type-categories can be compared with the expectations under Hardy-Weinberg equilibrium. In 1999, the estimated frequency of *I* was 0.84, and chi-square analysis, using the data in Table 2, indicates that the observed numbers do not differ from those expected under Hardy-Weinberg equilibria at $P = 0.50$ ($\chi^2 = 3.09$, df = 4). Although this is not a particularly powerful test due to the large number of assumptions (10), it does lend support to the hypothesis that this population meets the criterion for panmixia.

Our results do not allow us to reject the null hypothesis that sexual recombination significantly impacts the structure of this population. It appears that sexual recombination plays a significant role in maintaining genotypic and gene diversity while concomitantly producing overwintering inoculum. Our data also suggest that sexual recombination may serve as a potent force for integrating a beneficial allele based on the finding that there were a total of 133 unique multilocus genotypes fully insensitive to mefenoxam between 1998 and 1999. An interesting question that can only be answered by following a fully sensitive population as it shifts to insensitivity is how much genetic diversity is lost, if any, during the PAF selection process? The question of how long mefenoxam resistance will remain in a population of *P. capsici* when selection pressure is removed can only be answered in a tentative way. It appears that in this population, insensitivity will not decrease within the time frame of a typical 2-year rotation and, once resistance to mefenoxam is established, the future usefulness of this fungicide may be extremely limited.

Comparison of the population structure reported at this single location is currently being compared with other locations in Michigan and the United States and should provide useful insight into the amount of genetic diversity in sensitive versus insensitive populations as well as the contribution of migration to *P. capsici* population structure.

TABLE 3. Clone contribution of 15 *Phytophthora capsici* isolates to the total number of isolates collected in 1999 ($N = 200$)

Isolate	No. of clones[a]	CT/MS[b]	No. of clones in early, mid, and late season[c]: 6/22 – 7/16 $N = 60$	7/20 – 8/3 $N = 80$	8/5 – 8/18 $N = 60$
JP571	2	A1/I	2	–	–
JP583	2	A1/I	2	–	–
JP944	3	A1/I	2	1	–
JP999	3	A1/I	2	1	–
JP1007	2	A1/I	1	1	–
JP1042	2	A2/I	1	1	–
JP1096	2	A1/I	–	1	1
JP1102	2	A2/I	–	2	–
JP1215	3	A2/I	3	–	–
JP1342	2	A2/IS	–	2	–
JP1369	2	A1/I	1	1	–
JP1384	4	A2/I	3	1	–
JP1512	2	A1/I	1	–	1
JP1555	3	A1/I	–	–	3
JP1632	40	A1/I	2	12	26

[a] Total number of isolates with identical multilocus amplified fragment length polymorphism profiles.
[b] CT = compatibility type and MS = mefenoxam sensitivity where S = sensitive, IS = intermediately sensitive, and I = insensitive as determined by in vitro screening on 100 ppm of mefenoxam-amended agar.
[c] Sample intervals based on sampling dates only.

ACKNOWLEDGMENTS

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Worksheet 3-A(12)(c). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. <u>You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative</u>. Use additional pages as need.

<u>For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed</u>. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: **Fungicides**

Study: **The spatiotemporal genetic structure of *Phytophthora capsici* in Michigan and implications for disease management.**

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted __X__
1b. Township caps _____
1c. Alternative not acceptable in consuming country _____
1d. Other (Please describe) _____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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Worksheet 3-A(12)(c). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. **Is the study on EPA's website?** Yes ______ No X

   1a. **If not on the EPA website, please attach a copy.**

2. **Author(s) or researcher(s)** K.H. Lamour
   M.K. Hausbeck

3. **Publication and Date of Publication** Phytopathology 92:681-684, 2002

4. **Location of research study** Michigan, USA

5. **Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.**
   Mefenoxam

6. **Was crop yield measured in the study?** Yes ______ No X

7. **Describe the effectiveness of the alternative in controlling pests in the study.**
   Insensitivity of *Phytophthora capsici* to mefenoxam, a commonly used fungicide, is common in Michigan fields. Insensitivity of the pathogen to this fungicide renders this treatment ineffective.

8. **Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?**
   The results of this study are directly applicable since the research was conducted in Michigan, USA.

Symposium

New Frontiers in Plant Disease Losses and Disease Management

The Spatiotemporal Genetic Structure of *Phytophthora capsici* in Michigan and Implications for Disease Management

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Root, crown, and fruit rot caused by *Phytophthora capsici* Leonian is a limiting factor for the production of peppers, tomatoes, and cucurbit crops in Michigan and the United States. Like many species in the genus *Phytophthora*, *P. capsici* has the potential for rapid polycyclic disease development from a limited amount of initial inoculum (6). *P. capsici* produces caducous sporangia that can be spread by wind-blown rain or release 20 to 40 motile zoospores in the presence of free water. The polycyclic phase of disease development is thought to be driven primarily by asexual spore dispersal at a local scale (within and down rows). Sexual reproduction requires both the A1 and A2 compatibility types (CTs) and results in the production of thick-walled oospores. Oospores are thought to serve as the primary survival structure outside of host tissue.

Recommended disease management strategies stress the importance of avoiding excess water in the plant rhizosphere by using well-drained fields, conservative irrigation, and planting on raised beds. Additional recommendations include rotation to nonsusceptible hosts for at least 2 years and the use of fungicides. The phenylamide fungicide (PAF) mefenoxam is a systemic compound with high activity against *P. capsici* and has been used by growers throughout the United States to control *P. capsici*. Insensitivity to PAF has been reported for a number of other oomycetous organisms (*Bremia lactucae*, *P. infestans*, and *P. sojae*, etc.) and appears to be conferred by a single incompletely dominant gene of major effect (1). Growers in Michigan practicing 2+-year rotation in well-drained fields using an array of fungicidal management tools have experienced significant losses to *P. capsici*. Michigan is the number one producer of cucumbers for pickling in the United States and it was at the request of grower groups associated with this industry that research into the epidemiology and reproductive biology of *P. capsici* on cucurbit hosts was initiated.

Although many researchers cite oospores as the most likely propagule for survival outside of host tissue, there have been very few investigations specifically aimed at determining the impact of sexual reproduction in natural populations of *P. capsici*. Our hypothesis was that the sexual stage may play an important role not only in survival but also in the adaptation of *P. capsici* populations to environmental stresses (e.g., fungicides). Our goal was to perform a comprehensive investigation of the phenotypic and genetic diversity present in *P. capsici* populations from the major vegetable production regions of Michigan, with the implicit intention of addressing questions concerning epidemiology, reproductive biology, and the durability of currently recommended management strategies.

METHODOLOGY

Isolate collection and maintenance. Sampling of diseased fields began at the end of the 1997 growing season and continued through September 2000. In all cases, fields were sampled on a grid with quadrants varying from 40 m^2 to 12 km^2. A limited number of isolates were collected in 1997. In 1998, the strategy was to collect as many samples from as many fields as possible. This strategy was modified in 1999 and 2000 to focus on specific fields. Isolations from diseased plants were made onto selective media and single zoospore cultures were generated according to standard single sporing techniques (3). Isolates were placed into long-term storage (15°C) using a hemp seed/sterile water technique.

Phenotypic characterization. Single zoospore isolates were screened for CT using known A1 and A2 isolates. In vitro screening techniques published for other *Phytophthora* species for assessing sensitivity to mefenoxam were compared and a novel, simple, high dose screen using 100 ppm of mefenoxam-amended V8 agar was found to separate field isolates into three modal distributions that appeared consistent with the expectations of a single incompletely dominant gene governing mefenoxam insensitivity (e.g., sensitive, intermediately sensitive, and fully insensitive). These putative mefenoxam sensitivity (MS) groupings were tested by performing a series of crosses and testing whether the observed progeny sets met the expectations for Mendelian inheritance of a single incompletely dominant gene controlling insensitivity to mefenoxam. Sexual crosses were conducted on unclarified V8 agar plates and incubated for 3 months in the dark. Individual germinated oospores were recovered after 3 months using previously published techniques (2).

The efficacy of this in vitro mefenoxam screening technique was further tested in pumpkin seedlings using progeny from a cross between parents intermediately sensitive to mefenoxam. Nine isolates from each of the three MS categories were screened for pathogenicity on untreated seedlings. Single sensitive, intermediately sensitive, and fully insensitive isolates were then placed onto the unwounded surface of plants treated with either a field rate of mefenoxam, three times the field rate, or distilled water. Lesion diameters on seedling stems were measured after 4 days.

Genetic characterization. Single zoospore isolates were grown in antibiotic-amended V8 broth for 3 days at room temperature. Mycelial mats were washed, frozen, lyophilized, and ground with a sterile mortar and pestle. DNA was extracted with either a Qiagen Dneasy extraction kit (Qiagen, Valencia, CA) or via a cetyltrimethylammonium bromide (CTAB) procedure. A variety

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of methods for generating molecular markers were tested for efficacy including isozyme, random amplified polymorphic DNA, and amplified fragment length polymorphism (AFLP). The AFLP technique resulted in a large number of reproducible markers and was chosen to characterize samples of *P. capsici* from Michigan. The AFLP technique involves cutting genomic DNA with moderately rare cutting (*Eco*RI) and frequent cutting (*Mse*I) restriction enzymes, while concomitantly ligating synthetic adaptor fragments of DNA to the sticky ends created by the restriction enzymes (7). The result is a large number of DNA fragments that have ends with known DNA sequences. Amplification of fragment subsets (termed fingerprints) can be accomplished using polymerase chain reaction (PCR) primers complementary to the adaptor sequences with additional "selective" nucleotides. Changing the amount and type of selective nucleotides results in different subsets or fingerprints. Stringent PCR cycling parameters (touchdown technique) are used to ensure the fidelity of the reaction. For the analysis summarized here, adaptor sequences and fluorescent labeled selective primers were purchased as a kit through Perkin-Elmer ABI (Applied Biosystems, Foster City, CA). Using this system, AFLP fragments were resolved on a polyacrylamide gel by an ABI 377 gene sequencer. Fluorescent labels were excited by a laser and band emissions were analyzed in the form of an electropherogram where peaks represent individual bands. The sizing of fragments was particularly robust because a DNA ladder was loaded with every sample into the gel. To test for the reproducibility of fingerprints, DNA was extracted from a single isolate on three separate occasions approximately 3 months apart and subjected to the aforementioned protocol.

Data analysis. Isolates with identical multilocus AFLP fingerprints were considered to be members of the same clonal lineage and only a single representative was used for analysis. Because AFLP markers can only be scored confidently for presence (1) or absence (0), allele frequencies were estimated based on the assumption that populations under investigation meet the criterion for Hardy-Weinberg equilibrium, and that loci have only one "present" allele. The term population refers to all samples taken from a single field during a single year.

Genetic diversity within single populations was assessed by calculating the average number of polymorphic bands and estimating the average heterozygosity. Fixation indices were calculated according to methods of Weir and Cockerham (8) for populations from the same site over multiple years and among populations in Michigan using the program tools for population genetic analysis (TFPGA) (M. P. Miller, Northern Arizona University, Flagstaff). Confidence intervals for F statistics at the 95% confidence level were generated by bootstrapping at 1,000 iterations. The program NTSYS-pc version 2.02k (Exeter Software, Setauket, NY) was used to construct a similarity matrix from the presence/absence (1/0) data. Cluster analysis using the unweighted pair group with arithmetic averages (UPGMA) method was performed on the matrix and a tree was generated to give a visual representation of isolate similarity. Excoffier's ARLEQUIN program (L. Excoffier, University of Geneva) was used to assess population differentiation using a phenetic approach termed analysis of molecular variance (AMOVA), which allows for total genetic variation to be partitioned within and among populations using a classical analysis of variance (ANOVA).

RESULTS

Phenotypic results. Five isolates were recovered in 1997 from five different farms (four A1 and one A2 CT). One isolate was fully insensitive to mefenoxam, whereas the other four were fully sensitive. These findings prompted the extensive sampling conducted in 1998 in which 523 isolates (473 from cucurbits and 30 from bell pepper) were collected from 14 farms. A frequency histogram plotting percent growth of control on 100 ppm of mefenoxam-amended media versus number of isolates revealed a trimodal distribution (3). Putative MS categories were assigned based on these groupings with sensitive (S) <30% growth of control, intermediately sensitive (IS) between 30 and 90% growth of control, and insensitive (I) >90% growth of control. In vitro crosses between isolates representative of the different putative sensitivity categories (S × S, I × S, IS × S, and IS × IS) resulted in progeny sets not significantly different than expected for insensitivity inherited as a single incompletely dominant gene unlinked to CT ($P = 0.05$) (3). In 1998, 55% of the isolates were sensitive to mefenoxam, 32% were intermediately sensitive, and 13% were fully insensitive to mefenoxam. A1 and A2 CTs were recovered in a ratio of approximately 1:1 in 8 of the 14 farms. Oospores were detected in naturally diseased cucurbit fruit from four farms, and 223 oospore progeny were recovered and germinated from a single diseased cucumber. All six possible MS × CT combinations were detected in this naturally occurring oospore progeny set (3).

In planta studies using sensitive, intermediately sensitive, and fully insensitive *P. capsici* isolates supported the in vitro screening categories, with sensitive isolates causing no disease on mefenoxam-treated plants, intermediately sensitive isolates being slowed by mefenoxam, and fully insensitive isolates showing no difference in the ability to colonize host tissue between treated and untreated plants at three times the field rate. All the progeny isolates were pathogenic on untreated pumpkin plants (K. H. Lamour and M. K. Hausbeck, *unpublished data*).

Sixty-three mefenoxam insensitive (18% intermediate and 82% fully insensitive) isolates were recovered from a single southwest Michigan field in 1998. Field experiments were conducted in this field during 1999 and 2000, testing alternative cultural control strategies, and no mefenoxam was applied. Two hundred isolates were recovered from this site over the course of the 1999 season and 34 isolates at the beginning of the 2000 season. Of the 200 isolates recovered in 1999 from this field, 141 had unique AFLP genotypes. Seventy percent of these were fully insensitive to mefenoxam, 28% were intermediately sensitive, and 2% were sensitive. In 2000, 15% of the isolates were intermediately sensitive and 85% were fully insensitive. A single fully insensitive clonal lineage rose in frequency over the course of the 1999 season and comprised 20% of the total number of samples recovered (4).

During 1999 and 2000, approximately 2,500 isolates were recovered from farms in Michigan. Both the A1 and A2 CTs were present in every field sampled, and mefenoxam insensitivity was detected in the majority of farms that had a history of mefenoxam use.

Genetic results. Nine populations from the four major vegetable production areas of Michigan were analyzed with the AFLP procedure ($N = 641$). AFLP analysis resolved a total of 94 clearly discernable markers when considering all the isolates together. No single isolate or group of isolates from a single location contained all 94 markers. The total number of AFLP loci in a single population ranged from 68 to 80. Seventeen (18%) fragments were fixed for the present state across all populations, 12 (13%) fragments were polymorphic in all populations, and 65 (69%) were fixed for presence or absence in some populations and polymorphic in others. The number of polymorphic bands within a single population ranged from 37 to 46 with estimated heterozygosities ranging from 0.18 to 0.22. Clonal reproduction was significant within single fields over the course of the growing season. For example, genotypic diversity in a single field ranged from 100% at the beginning of the growing season (seedling stage) to <30% at the time cucurbit fruit were ready for harvest (4). When considering all nine populations, genotypic diversity ranged from 42 to 96% with an average of 74% of the isolates in any sample set having unique genotypes. Although clonal reproduction was significant within single fields within years, no clones were recovered from single fields between years or among fields separated by at least 1 km. Fixation indices (ϕ_{ST}) between the

populations sampled on consecutive years were very close to zero, indicating that gene diversity was not measurably impacted by genetic drift (5). The overall estimated ϕ_{ST} for populations from different locations was 0.35, indicating that approximately 35% of the total genetic diversity present in Michigan *P. capsici* populations is found among populations and 65% is found within any one population. AMOVA partitioned genetic diversity among (40%) and within (60%) populations. The similarity tree based on UPGMA cluster analysis clearly showed that isolates from the same site sampled over years branched from the same node, with no clustering of isolates based on the year of sampling. Cluster analysis also clearly showed that populations separated geographically branched from population-specific nodes (5).

DISCUSSION

During the past 10 years, Michigan has experienced a steady increase in the incidence of root, fruit, and crown rot on cucurbits

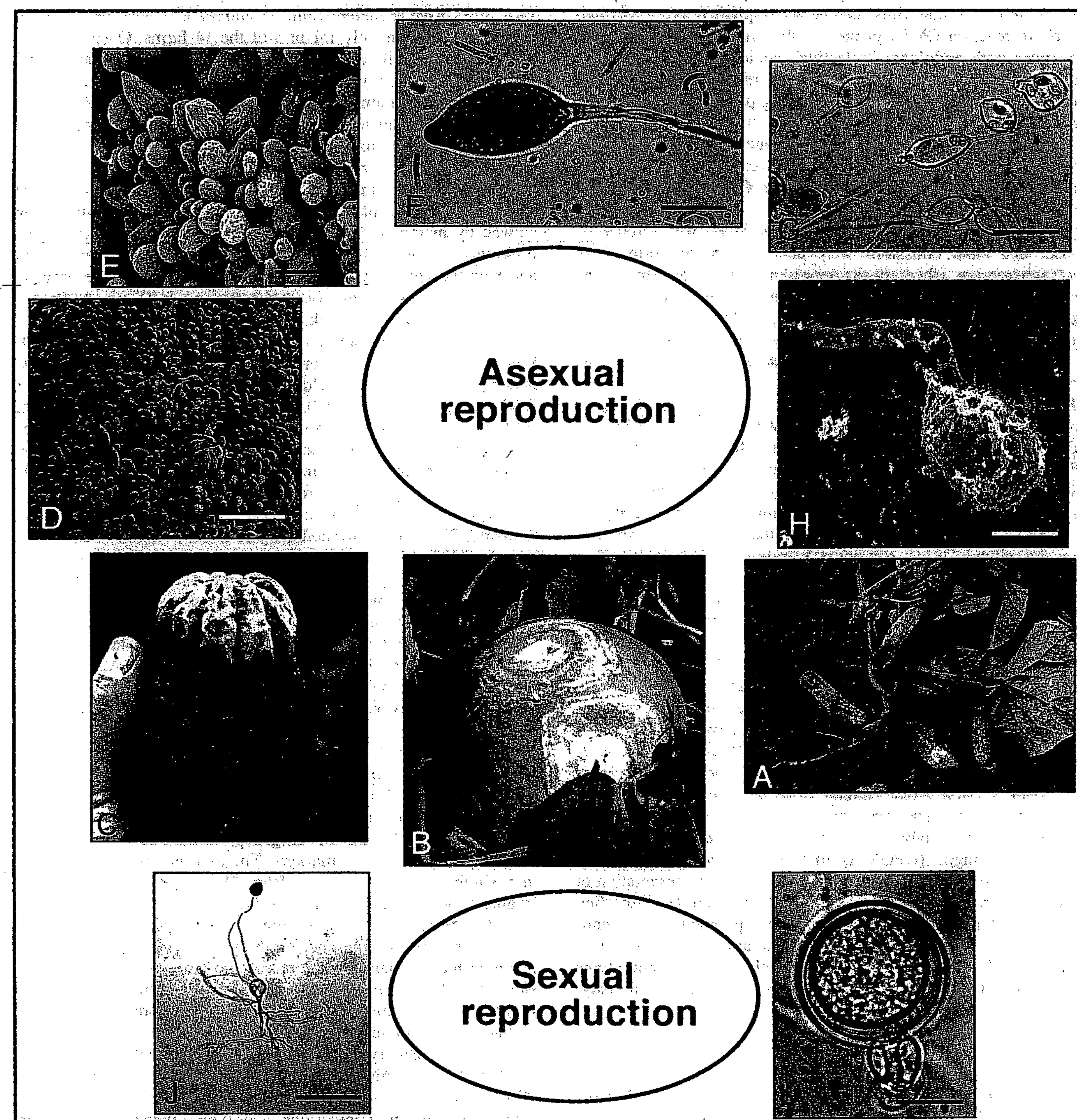

Fig. 1. Spore types and signs of infection caused by *Phytophthora capsici* on cucurbit fruit: **A,** infected cucumber, **B,** pumpkin, and **C,** acorn squash fruit. **D,** Scanning electron microscope (SEM) photo of an infected cucumber showing tufts of sporangia produced on the surface of the fruit (Bar = 300 µm). **E,** Close-up of a single tuft of sporangia (Bar = 30 µm). **F,** Typical papillate sporangium with a long pedicel (Bar = 20 µm). **G,** Zoospores exiting sporangia after immersion in water (Bar = 50 µm). **H,** SEM photo of a single encysted zoospore that germinated and directly penetrated the epidermis of a cucumber fruit (Bar = 4 µm). **I,** Typical amphigynous oospore (Bar = 10 µm). **J,** A germinating oospore with multiple germ tubes and a terminal sporangium (Bar = 100 µm).

caused by *P. capsici*. Rotation to nonsusceptible hosts, in conjunction with cultural and chemical control strategies, have not provided economic control. Correspondence with other vegetable pathologists suggests that this phenomenon is not confined to Michigan, and a similar increase in control failures due to blight by *P. capsici* is being reported throughout the United States.

Investigation of the inheritance of MS demonstrated that MS is inherited as a single incompletely dominant gene unlinked to CT. In 1998, all six possible MS × CT combinations were present in single fields and insensitivity to mefenoxam was common in Michigan. Typical amphigynous oospores were observed in *P. capsici*-infected cucurbit fruit from multiple locations, and oospore progeny from a single naturally infected fruit showed segregation for MS and CT. These findings strongly support the hypothesis that sexual reproduction is occurring in the field, and also suggest that sexual recombination may directly generate progeny fully insensitive to mefenoxam. Tracking a single mefenoxam insensitive population over 2 years in the absence of mefenoxam selection pressure suggests that costs associated with mefenoxam insensitivity are minimal.

Estimates of average heterozygosity and polymorphism indicate surprisingly high levels of gene and genotypic diversity in all the populations of *P. capsici* analyzed. Tracking a single population through an entire growing season showed that asexual reproduction plays a significant role in disease development within a single season. Sampling single fields over consecutive years suggested that clones do not survive Michigan winters and that oospores are the primary survival propagule. Estimation of fixation indices for samples from the same site over consecutive years suggested that there was not a significant reduction in genetic diversity between growing seasons. This implies that populations are large enough to withstand dramatic effects of genetic drift. Cluster analysis revealed unambiguous groups corresponding to geographical locations with regional populations showing more similarity overall than populations from different regions. Population pairwise fixation indices corroborated this finding. The estimated overall fixation index and AMOVA are in agreement with both, suggesting that most (approx 60%) of the total genetic variability in Michigan is found within any one population, but that a relatively large component (40%) of genetic variability is found among populations.

Recommendations based on our findings are as follows: (i) the fungicide mefenoxam may be of limited usefulness because insensitivity appears to be selected for rapidly and is unlikely to decrease when mefenoxam selection pressure is removed; (ii) fields with epidemics are likely to harbor oospores for an extended amount of time (at least 5 years), and this factor must be considered before replanting to susceptible hosts; and (iii) factors that may contribute to the introduction of *P. capsici* into uninfested fields (e.g., drainage ditches between farms, irrigation ponds, and the dumping of culls) need to be considered and if possible avoided, because once an epidemic is established we have found no evidence that the population will become extinct in an agriculturally meaningful time period.

From an evolutionary perspective, it is clear that *P. capsici* has successfully colonized a number of geographical locations in Michigan and that each of the populations sampled thus far have similarly high levels of genetic variability. The genetic stability of single populations over multiple years, the high fixation indices between even geographically close populations (1 km), and the clear structuring based on UPGMA cluster analysis all suggest that long-distance dispersal of inoculum is not common and that geographically isolated populations are also genetically isolated. It appears that the sexual stage of the *P. capsici* life cycle plays a significant role in survival as well as maintaining both genic and genotypic diversity, and has likely played a key role in the evolution of mefenoxam insensitivity. The combination of high levels of genetic variability, thick-walled oospores, and polycyclic asexual disease development make *P. capsici* a formidable pathogen (Fig. 1). This work underscores the need for management strategies aimed at preventing the spread of *P. capsici* to uninfested field sites and suggests that management strategies aimed at limiting spread within a single season may be the only option for growers with *P. capsici*-infested fields.

ACKNOWLEDGMENTS

This work was funded by the Michigan Agricultural Experiment Station, Michigan State University Extension, Michigan Department of Agriculture, Michigan Farm Bureau (GREEEN cooperative), Pickle and Pepper Research Committee, Pickle Packers International, Inc., and the Pickle Seed Research Fund, Pickle Packers International. We thank M. Bour, C. Hunter, J. Jabara, P. Tumbalam, E. Webster, and J. Woodworth for competent laboratory assistance. K. Lamour thanks his Ph.D. committee members A. Jarosz, R. Hammerschmidt, and F. Trail for guidance and extends sincere thanks to M. Hausbeck for fulfilling her role as mentor in an exemplary manner.

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Worksheet 3-A(12)(d). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative. Use additional pages as need.

For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Fungicides **Study: Evaluation of selected fungicides for control of Phytophthora blight of pepper, 2000.**

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted ___X___
1b. Township caps ______
1c. Alternative not acceptable in consuming country ______
1d. Other (Please describe) ______

______

______

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

Worksheet 3-A(12)(d). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. **Is the study on EPA's website?** **Yes** ______ **No** X

   1a. **If not on the EPA website, please attach a copy.**

2. **Author(s) or researcher(s)** M. Babadoost
   J.D. Kindhart

3. **Publication and Date of Publication** Fungicide and Nematicide Tests 56:V31, 2001

4. **Location of research study** Michigan, USA

5. **Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.**
   Ridomil Gold/Copper
   Acrobat MZ
   Actigard

6. **Was crop yield measured in the study?** **Yes** ______ **No** X

7. **Describe the effectiveness of the alternative in controlling pests in the study.**
   None of the fungicides tested adequately controlled *Phytophthora capsici* throughout the growing season.

8. **Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?**
   Michigan growers would expect similar results, because the disease pressure, crop production system, and weather conditions are comparable to that observed in Illinois.

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EVALUATION OF SELECTED FUNGICIDES FOR CONTROL OF PHYTOPHTHORA BLIGHT OF PEPPER, 2000: The objective of this experiment was to determine if Phytophthora blight of pepper, caused by *Phytophthora capsici*, could be controlled by application of fungicides. Field experiments were conducted in a commercial field near Shawneetown and at the Dixon Springs Agricultural Center, Simpson, IL. The commercial field was naturally infested with *P. capsici* in 1999, but the experimental site at Dixon Springs was only suspected to be infested with *P. capsici*. Five-week-old seedlings were transplanted on 30 May into raised beds with drip irrigation. Plots consisted of 10 plants in two staggered rows with plants spaced 12 in. apart within and between rows. The plots were spaced 36 in. apart in a completely randomized block design with three replications. Weeds were controlled by hand weeding. A liquid fertilizer of N-P-K (4-0-8) was injected into irrigation system once per week. Plants received daily drip irrigation. Acrobat MZ, Actigard, and USF 2001 were foliar sprayed; Ridomil Gold/Copper was applied as soil-drench and foliar-spray; and Ridomil Gold EC was applied as soil-drench. Actigard was applied onto the seedlings 7 and 1 day prior to transplanting and at 10-day intervals thereafter. All other chemicals were applied as indicated on the table. All of the chemicals were applied with a backpack sprayer, using 30 gal of water per acre. Average monthly high and low temperatures (F) were 86/65, 94/55, and 89/69 in Jun, Jul, and Aug, respectively. Disease incidence was determined as percent wilted and dead plants on 28 Jun, 6 Jul, 26 Jul, and 5 Aug.

No Phytophthora infection was detected in the trial at Dixon Spring Agricultural Center. Plants in the field near Shawneetown became severely infected. None of the chemicals used provided complete protection for the plants against *P. capsici*. However, Acrobat MZ and Actigard appeared to have promising potential for reducing the incidence of Phytophthora blight of pepper in the field. In spite of the highly conducive environmental conditions for Phytophthora blight and high disease pressure, more than 50% of plants treated with Acrobat MZ and Actigard survived through the season.

	Phytophthora disease incidence (%)[1]				
Treatment and rate/A (application)[2]	28 Jun	6 Jul	16 Jul	26 Jul	5 Aug
Control	6.7	23.3	73.3	73.3	83.3
Acrobat MZ (F)[3] 2.25 lb (2-5)	0.0	6.7	30.0	40.0	43.3
Acrobat MZ (F)+Ridomil Gold/Copper (F) 2.25 lb+2.5 lb (2-5+2-5)	10.0	10.0	36.7	43.3	53.3
Acrobat MZ (F)+Ridomil Gold/EC (S)[3] 2.25 lb+1.0 pt (2-5+2,3)	10.0	23.3	36.7	40.0	43.3
Actigard 50WG (F) 1.0 oz. (0-5)	3.3	13.3	30.0	33.3	46.7
Actigard 50WG (F)+Ridomil Gold/Copper (F) 1.0 oz.+2.5 lb (2-5+2-5)	0.0	30.0	46.7	60.0	60.0
Actigard 50WG (F)+Ridomil Gold/EC (S) 1 oz.+1.0 pt (2-5+2,3)	0.0	13.3	43.3	46.7	46.7
Ridomil Gold/Copper (F) 2.5 lb (2-5)	13.3	40.0	50.0	76.7	100
Ridomil Gold/Copper (F+S) 2.5 lb (2-5+2,3)	6.7	23.3	50.0	56.7	70.0
Ridomil Gold/EC (S) 1.0 pt (1-3)	3.3	26.7	56.7	70.0	86.7
USF-2001 520SC (F) 6.0 fl. oz. (2-5)	3.3	30.0	53.3	70.0	83.3
USF-2001 520SC(F)+Ridomil Gold/Copper (F) 6 fl. oz. +2.5 lb (2-5+2-5)	0.0	20.0	43.3	53.3	73.3
USF-2001 520SC(F)+Ridomil Gold/EC (S) 6.0 fl. oz.+1.0 pt (2-5+2,3)	0.0	26.7	60.0	63.3	76.7
LSD ($P \leq 0.05$)	12.3	29.7	47.6	50.2	45.2

[1] Percent plant infected with *Phytophthora*. Each value represents the mean of 3 replications.
[2] Application time: 0=24 May, 1=30 May, 2=28 Jun, 3=6 Jul, 4=16 Jul, 5=28 Jul.
[3] F=foliar application; S=soil application.

Worksheet 3-A(13). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative. Use additional pages as need.

For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Steam **Study:** UNEP 1998, B-83, B-86, B-90, B-282

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted X
1b. Township caps ____
1c. Alternative not acceptable in consuming country ____
1d. Other (Please describe) ____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

Worksheet 3-A(13). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. **Is the study on EPA's website?** **Yes** X **No** ______

   1a. **If not on the EPA website, please attach a copy.**

2. **Author(s) or researcher(s)** ______________________

3. **Publication and Date of Publication** ______________________

4. ***Location of research study*** ______________________

5. **Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.**

   Steam

6. **Was crop yield measured in the study?** **Yes** ______ **No** ______

7. **Describe the effectiveness of the alternative in controlling pests in the study.**

   ____

8. **Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?**

   In the studies referenced, steaming has been used in protected production systems, such as greenhouses. The use of steam has not proven economical and practical when large, unprotected areas are treated. In Michigan systems, *Phytophthora capsici* has an airborne spore that would render the use of steam ineffective.

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Worksheet 3-A(14). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative. Use additional pages as need.

For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

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The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Biological Control **Study:** UNEP 1998, B-83, B-87, B-91, B-92, B-285, B-287, B-45

Section I. Initial Screening on Technical Feasibility of Alternatives

1. **Are there any location-specific restrictions that inhibit the use of this alternative on your site?**

   1a. Full use permitted X

   1b. Township caps ____

   1c. Alternative not acceptable in consuming country ____

   1d. Other (Please describe) ____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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Worksheet 3-A(14). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes X No ____

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ______________

3. Publication and Date of Publication ______________

4. Location of research study ______________

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

   Biological Control

6. Was crop yield measured in the study? Yes ____ No ____

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?

   In the studies described, *Phytophthora capsici* was not a target pathogen, so they do not apply to Michigan's situation.

Worksheet 3-A(14)(b). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative. Use additional pages as need.

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When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

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(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Biological Control **Study:** Alternatives for methyl bromide on cucurbits and solanaceous crops, 2002.

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted ___X___
1b. Township caps ______
1c. Alternative not acceptable in consuming country ______
1d. Other (Please describe) ______

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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Worksheet 3-A(14)(b). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. **Is the study on EPA's website?** **Yes** ______ **No** X

   1a. If not on the EPA website, please attach a copy.

2. **Author(s) or researcher(s)** M.K. Hausbeck
   B.D. Cortright

3. **Publication and Date of Publication** Research in progress

4. **Location of research study** Michigan, USA

5. **Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.**
   Multigard FFA, Multigard Protect, Multigard Protect + Vapam HL, CX-100

6. **Was crop yield measured in the study?** **Yes** ______ **No** X

7. **Describe the effectiveness of the alternative in controlling pests in the study.**
   Fields have not been harvested yet.

8. **Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?**

   The results of this study are directly applicable, since the research was conducted in Michigan, USA.

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Worksheet 3-A(15). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative. Use additional pages as need.

For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Cover Crops, Mulching **Study:** UNEP 1995, UNEP 1998, A-74, A-77, B-91, B-284, A-66, B-42, B-287

Section I. Initial Screening on Technical Feasibility of Alternatives

1. **Are there any location-specific restrictions that inhibit the use of this alternative on your site?**

1a. Full use permitted	X
1b. Township caps	
1c. Alternative not acceptable in consuming country	
1d. Other (Please describe)	

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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Worksheet 3-A(15). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes __X__ No ______

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ______________

3. Publication and Date of Publication ______________

4. Location of research study ______________

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

   Cover Crops, Mulching

6. Was crop yield measured in the study? Yes ______ No ______

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?

   The results of the study are not relative to the situation in Michigan, because the examples provided specifically discuss control of weeds and nematodes. The only pathogen included was *Sclerotinia sclerotiorum*. Michigan growers are managing *Phytophthora capsici* currently using black plastic mulch, but it is not a viable alternative alone to control this pathogen.

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Worksheet 3-A(16). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative. Use additional pages as need.

For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
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Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.
The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Crop Residue Compost **Study:** UNEP 1998, B-40

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?
   - 1a. Full use permitted X
   - 1b. Township caps ____
   - 1c. Alternative not acceptable in consuming country ____
   - 1d. Other (Please describe) ____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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Worksheet 3-A(16). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes X No ____

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ______________

3. Publication and Date of Publication ______________

4. Location of research study ______________

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

   Crop Residue Compost

6. Was crop yield measured in the study? Yes ____ No ____

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?

   The results of this study do not apply to Michigan, because they were not tested against *Phytophthora capsici*, a primary pathogen in Michigan, USA. Also, the degree of efficacy in using compost product to control soil-borne pathogens vary regionally, so that composts that control pathogens in one region may not do so in another region.

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Worksheet 3-A(17). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. <u>You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative</u>. Use additional pages as need.

<u>For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed</u>. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
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(3) Cite research listed on the EPA website

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The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Crop Rotation, Fallow **Study:** UNEP 1995, UNEP 1998, UNEP 2001, A-73, B-83, B-87, B-93, B-94, B-99, B-282, E-74, B-79

Section I. Initial Screening on Technical Feasibility of Alternatives

1. *Are there any location-specific restrictions that inhibit the use of this alternative on your site?*

1a. Full use permitted ___X___
1b. Township caps ______
1c. Alternative not acceptable in consuming country ______
1d. Other (Please describe) ______

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If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

For EPA Use Only
ID# ______________

Worksheet 3-A(17). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes __X__ No ____

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ______________

3. Publication and Date of Publication ______________

4. Location of research study ______________

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

   Crop Rotation, Fallow

6. Was crop yield measured in the study? Yes ____ No ____

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?

   Rotation to crops not susceptible to crown, root and fruit rot caused by *Phytophthora capsici* is practiced routinely by growers of solanaceous crops in Michigan. This management practice is not adequate, because of the long-lived oospore of this pathogen. Since many other vegetable crops are also susceptible, including all cucurbit crops and beans (new report of lima beans as a host), this would make rotation difficult even if it was effective. Crop rotation and fallow is not a suitable alternative to manage *P. capsici* on solanaceous crops in Michigan.

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ID# ______________

Worksheet 3-A(17)(b). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. <u>You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative</u>. Use additional pages as need.

<u>For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed</u>. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

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The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Crop Rotation, Fallow **Study: Investigating the spatiotemporal genetic structure of *Phytophthora capsici* in Michigan.**

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted ___X___
1b. Township caps ______
1c. Alternative not acceptable in consuming country ______
1d. Other (Please describe) ______

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If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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Worksheet 3-A(17)(b). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. **Is the study on EPA's website?** **Yes** ______ **No** X

    1a. **If not on the EPA website, please attach a copy.**

2. **Author(s) or researcher(s)** K.H. Lamour
   M.K. Hausbeck

3. **Publication and Date of Publication** Phytopathology 91:973-980, 2001

4. **Location of research study** Michigan, USA

5. **Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.**
   Crop Rotation

6. **Was crop yield measured in the study?** **Yes** ______ **No** X

7. **Describe the effectiveness of the alternative in controlling pests in the study.**
   Crop rotation is not highly effective because both mating types of *Phytophthora capsici* are present in Michigan fields, resulting in an oospore capable of surviving for long period of time.

8. **Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?**
   Results are directly applicable, since the research was conducted in Michigan.

Investigating the Spatiotemporal Genetic Structure of *Phytophthora capsici* in Michigan

K. H. Lamour and M. K. Hausbeck

Department of Plant Pathology, Michigan State University, East Lansing 48824.
Accepted for publication 1 July 2001.

ABSTRACT

Lamour, K. H., and Hausbeck, M. K. 2001. Investigating the spatiotemporal genetic structure of *Phytophthora capsici* in Michigan. Phytopathology 91:973-980.

Phytophthora capsici isolates were recovered from pepper and cucurbit hosts at seven locations in Michigan from 1998 to 2000. Isolates were characterized for compatibility type (CT), mefenoxam sensitivity (MS), and amplified fragment length polymorphism (AFLP) marker profiles. In total, 94 AFLP bands were resolved. Individual populations were highly variable. Within populations, 39 to 49% of the AFLP bands were polymorphic and estimated heterozygosities ranged from 0.16 to 0.19. Of the 646 isolates fingerprinted, 70% (454) had unique AFLP profiles. No clones were recovered between years or locations. Pairwise *F* statistics (Φ_{ST}) between populations from different locations ranged from 0.18 to 0.40. A tree based on unweighted pair-group method with arithmetic average cluster analysis indicates discrete clusters based on location. Isolates from the same location showed no clustering based on the year of sampling. Analysis of molecular variance partitioned variability among (40%) and within populations (60%). The overall estimated Φ_{ST} was 0.34 (SD = 0.03). A1/A2 CT ratios were ≈1:1, and MS frequencies were similar between years for the two locations sampled over time. These data suggest that *P. capsici* persists in discrete outcrossing populations and that gene flow among locations in Michigan is infrequent.

Phytophthora capsici Leonian causes significant damage to a variety of plant hosts worldwide, and in the United States, it seriously impacts the production of cucurbits, tomatoes, and peppers (9,14,20). In Michigan, the life history of *P. capsici* is divided between an active growth phase in the presence of susceptible host tissue and a state of dormancy over the winter. Overwintering survival is thought to be accomplished by thick-walled oospores that are produced during sexual reproduction (9,10). *P. capsici* is heterothallic, and completion of the sexual stage requires both A1 and A2 compatibility types (CT). Sexual reproduction is mediated by extracellular hormonal signals, and there is the potential for both self and cross-fertilization (8). Oospores generally require a dormancy period prior to germination. Germinating oospores produce coenocytic mycelium, which can directly infect or differentiate into caducous sporangia under suitable conditions. Sporangia can be dislodged and cause infection directly, or, in the presence of free water, release 20 to 40 motile zoospores. Polycyclic asexual spread of *P. capsici* between and down rows has been clearly documented in the pepper/*P. capsici* pathosystem (21).

Ristaino and Johnston recently summarized management strategies useful for disease control (20). The primary strategy is to manage soil water dynamics by providing the best possible drainage for the host plant's rhizosphere and the field, in general. Growers are advised to rotate fields to nonsusceptible hosts, and when appropriate to apply fungicides.

The phenylamide fungicide mefenoxam is fungistatic to sensitive isolates of *P. capsici* (16), but, as has occurred with many oomycetes, insensitivity has developed in field populations (9,17,18). Research with *P. capsici* isolates from Michigan indicates that insensitivity is controlled by an incompletely dominant gene of major effect (9), which is consistent with the findings for other oomycetes (3).

In Michigan, fruit, stem, and root rots caused by *P. capsici* on susceptible hosts have increased in recent years, and growers employing available management strategies have experienced significant losses. Over the last 4 years, an investigation of *P. capsici* populations in Michigan commercial vegetable production fields has been conducted (9,10). The initial phase of this study was based on the distribution and frequency of CT and mefenoxam sensitivity (MS) phenotypes within fields. In 1998, an approximate 1:1 ratio of A1/A2 isolates was discovered in the majority of fields sampled, and oospores were detected in diseased cucurbit fruit on four separate farms. All six CT/MS phenotypes were recovered as oospore progeny from a single diseased cucumber fruit as well as from a single diseased cucumber field (9). These initial findings suggested that the sexual stage occurs in populations of *P. capsici* in Michigan and, based on the MS findings, that sexual recombination may play an important role in generating the fully insensitive MS phenotype.

The ability to assess population structure by only CT and MS is limited by the fact that only six phenotypic combinations are resolvable and is further limited because some populations appear to have only sensitive or insensitive isolates (9). Amplified fragment length polymorphism (AFLP) markers are increasingly used as a tool to investigate population genetic structure in a wide variety of living organisms including plants (22,24), animals (19), insects (4), and microorganisms (11). A molecular map of the *P. infestans* genome was constructed based on AFLP and restriction fragment length polymorphism markers and corroborates the finding of other researchers that AFLP markers span the genome (23). The recent characterization of a single mefenoxam-insensitive population of *P. capsici* with AFLP markers over a 2-year period revealed that genotypic and genic diversity were high, that clonal reproduction (the recovery of identical multilocus genotypes from different locations within a field) was significant within a single season but that members of the same clonal lineage were not detected between years, that AFLP marker frequencies did not

Corresponding author: K. H. Lamour; E-mail address: lamourku@msu.edu

Publication no. P-2001-0725-01R

change significantly between years, and that the frequency of mefenoxam insensitivity did not appear to decrease in the absence of mefenoxam use (10).

In this paper, we report on the genetic structure of *P. capsici* populations from fields located in different regions of Michigan. It was our goal to consider dispersal between locations and the impact of outcrossing on natural populations. We also report on the frequency of self-fertilized versus outcrossed progeny in a sexual cross between isolates from different geographical locations and the inheritance of AFLP markers in this cross. Portions of the information in this paper have been reported previously (9,10).

MATERIALS AND METHODS

Isolate collection and maintenance. Pepper, cucumber, pumpkin, tomato, and squash plant tissue (root, crown, and fruit) with typical signs and symptoms of infection by *P. capsici* were collected from six farms in four different regions of Michigan between 1998 and 2000. Sampling was conducted using field-specific grids with grid quadrants varying from 40 m^2 to 12 km^2, depending on the size of the field. Sample sets are labeled according to the following notational approach: location (SW = southwest, SC = south central, C = central, and NW = northwest) followed by a farm designation (1,2,...*n*) with a hyphen separating a field designation (A,B,...*n*) and the year sampling was conducted (98, 99, and 00). Diseased plant tissue (between 4 to 20 per quadrant) was collected from quadrants in an arbitrary fashion. Isolation from diseased plant material was made onto BARP (25 ppm of benomyl, 100 ppm of ampicillin, 30 ppm of rifampicin, and 100 ppm of pentachloronitrobenzene)-amended UCV8 (840 ml of distilled water, 163 ml of unclarified V8 juice, 3 g of $CaCO_3$, and 16 g of agar) plates. Procedures for obtaining single zoospore isolates were as previously described (9). Single zoospore cultures were maintained on RA (30 ppm of rifampicin and 100 ppm of ampicillin)-UCV8 plates and transferred bimonthly. For long-term storage, a 7-mm plug of expanding mycelium from each culture was placed in a 1.5-ml microfuge tube with one sterilized hemp seed and 1 ml of sterile distilled water (SDW). Isolates were then incubated for 2 to 3 weeks at 23 to 25°C before being stored at 15°C.

CT and MS determination. Agar plugs from the edge of an expanding single zoospore colony were placed at the center of UCV8 plates approximately 2 cm from ATCC isolate 15427 (A1 CT) and ATCC 15399 (A2 CT) and incubated in the dark at 23 to 25°C for 3 to 6 days. Following incubation, CT was determined. Thereafter, all CT determinations were crossed with field isolates OP97 (A1) and SP98 (A2).

Agar plugs from the edge of actively expanding single zoospore colonies were placed at the center of UCV8 plates (100 × 15 mm) amended with 0 or 100 ppm of mefenoxam (Ridomil Gold EC, Novartis, Greensboro, NC; 48% active ingredient, suspended in SDW; added to UCV8 cooled to 49°C). Inoculated plates were incubated at 23 to 25°C for 3 days, and colony diameters were measured. Percent growth of an isolate on amended media was calculated by subtracting the inoculation plug diameter (7 mm) from the diameter of each colony and dividing the average diameter of the colony on amended plates by the average diameter of the colony on unamended control plates. All tests were conducted at least two times. An isolate was scored as sensitive (S) if growth at 100 ppm was <30% of the control, intermediately sensitive (IS) if growth was between 30 and 90% of the control, and insensitive (I) if growth was >90% of the control (9).

DNA extraction and AFLP fingerprinting. Bacterial contamination was avoided by using a modified Van Teigham cell (5). The uppermost portion of a 7-mm plug of mycelium was placed on the surface of RA-WA plates (30 ppm of rifampicin, 100 ppm of ampicillin, 1,000 ml of distilled water; and 16 g of agar) and an autoclaved cap from a 1.5-ml microfuge tube was placed over the plug, which forced the isolate to grow through the amended medium. Isolates were incubated in the dark for 2 to 3 days before two 7-mm plugs were transferred to approximately 15 ml of RA-UCV8 broth in petri dishes (100 × 5 mm) and incubated in the dark for 3 days at 23 to 25°C. Mycelial mats were washed with distilled water and dried briefly under vacuum before being frozen to –20°C and lyophilized.

Lyophilized mats were ground with a sterile mortar and pestle. Whole genomic DNA from approximately 50 mg of ground mycelium was extracted with a plant mini kit (Qiagen Dneasy; Qiagen Inc., Valencia, CA) according to the manufacturer's directions or using a cetyltrimethylammonium bromide (CTAB) procedure in conjunction with an automated DNA extractor. DNA was

TABLE 1. Inheritance of 17 amplified fragment length polymorphism (AFLP) markers, compatibility type (CT), and mefenoxam sensitivity (MS) in 107 progeny of a cross between *Phytophthora capsici* isolates OP97 (A1/IS) and SFF3 (A2/S)

Marker[a]	Progeny ratio[b]	χ^{2c}	P[d]
E+AC/M+CA-66	47:60	1.58	0.20
E+AC/M+CA-97	51:56	0.23	0.70
E+AC/M+CA-146	53:54	0.01	0.90
E+AC/M+CA-149	60:47	1.58	0.20
E+AC/M+CA-156	64:43	4.12	0.04
E+AC/M+CA-159	56:51	0.23	0.70
E+AC/M+CA-244	46:61	2.10	0.17
E+AC/M+CA-258	52:55	0.08	0.80
E+AC/M+CA-270	53:54	0.01	0.98
E+AC/M+CA-282	56:51	0.23	0.70
E+AC/M+CA-290	62:45	2.70	0.13
E+AC/M+CA-328	55:52	0.08	0.80
E+AC/M+CA-351	61:46	2.10	0.15
E+AC/M+CA-398	55:52	0.08	0.80
E+AC/M+CA-431	55:52	0.08	0.80
E+AC/M+CA-435	57:50	0.46	0.90
E+AC/M+CA-444	49:58	0.76	0.85
CT	53:54	0.01	0.98
MS	47:60	1.58	0.20

[a] AFLP marker labels indicate the restriction enzymes (E = *Eco*RI, M = *Mse*I), the two selective nucleotides, and the size of the DNA fragment in base pairs.
[b] Presence/absence ratios for AFLP markers, A1/A2 for CT, and sensitive (S)/intermediately sensitive (IS) for MS as determined by in vitro screening.
[c] χ^2 value for testing 1:1 segregation (1 df).
[d] Probability of the observed ratio occurring by chance under the null hypothesis of 1:1 segregation.

TABLE 2. Estimates of genetic diversity within populations of *Phytophthora capsici* in Michigan

Population[a]	No. of isolates[b]	No. of AFLP bands	No. and % polymorphic bands[c]	Estimated average heterozygosity
SW1-A98	57	72	37 (39)	0.16
SW1-A99	141	72	37 (39)	0.16
SW1-B99	35	69	38 (40)	0.16
SW1-B00	24	69	38 (40)	0.16
SC1-A98	50	68	42 (45)	0.17
SC2-B99	45	71	43 (46)	0.17
C1-A00	48	77	41 (44)	0.17
NW1-A99	37	80	44 (47)	0.19
NW2-B98	24	73	46 (49)	0.18

[a] First two capital letters indicate location in Michigan with S = south, W = west, C = central, and N = north, the number following the location designator indicates the farm, the capital letter following the hyphen is a field designator, and the numbers following the field designator indicate year (e.g., 00 = 2000).
[b] Total number of isolates with unique multilocus amplified fragment length polymorphism (AFLP) profiles.
[c] Percent polymorphic bands determined by dividing the number of polymorphic bands by the total number of bands recovered in Michigan (N = 94).

quantified by Nucleic Acid QuickSticks (Clontech, Palo Alto, CA) according to the manufacturer's directions or on 1.5% agarose gels. Approximately 100 ng of DNA was subjected to a restriction/ligation reaction, preselective amplification, and selective amplifications using the polymerase chain reaction (PCR) core mix, adaptor sequences, core primer sequences, and fluorescence-labeled primers provided by an AFLP microbial fingerprinting kit (Perkin-Elmer Applied Biosystems [PE/ABI], Foster City, CA) and performed exactly as described in the AFLP microbial fingerprinting protocol part 402977 Rev A (PE/ABI) (25). All PCR reactions were performed with a minicycler (MJ Research Inc., Waltham, MA) in 0.2-ml tubes according to the cycling parameters outlined in the microbial fingerprinting protocol.

An initial optimization set of reactions was performed with preselective products from *P. capsici* isolate OP97, which was isolated from a cucumber fruit in 1997 (9). Selective amplifications with the selective primers *Eco*RI-AA, AC, AG, and AT were performed in all 16 combinations with the *Mse*I-CA, CC, CG, and CT selective primers. *Eco*RI-selective primers available from PE/ABI were labeled at the 5′ end with either carboxyfluorescein (FAM), carboxytetramethyrhodamine (TAMRA), or carboxy-4′,5′-dichloro-2′,7′-dimethoxyfluorescein (JOE) fluorescent dyes. The fluorescent dyes were excited by laser radiation and visualized by their characteristic absorption-emission frequencies. Only the fragments containing an *Eco*RI restriction site were resolved.

Selective amplification AFLP products and a carboxy-X-rhodamine size standard were loaded into each lane on a denaturing polyacrylamide gel and the fragments resolved in a DNA sequencer (Prism 377; ABI). Results were prepared for analysis in the form of electropherograms using GeneScan Analysis software (PE/ABI). AFLP fragments were scored manually as present = 1 or absent = 0 using Genotyper (PE/ABI). Only DNA bands that consistently exhibited unambiguous presence/absence profiles were scored.

In order to assess the reproducibility of AFLP profiles, a single isolate, OP97, was subjected to the aforementioned protocol using three optimal primer pair combinations on three separate occasions approximately 3 months apart.

No prior sequencing or cloning of fragments is needed to utilize this marker system and it is highly reproducible between labs (1). AFLP markers are generally scored as present or absent (e.g., dominant markers), and the confidence with which population level inferences can be made is greatly increased by sample sets that are approximately twice the size used for codominant markers (7,12,28).

Marker inheritance. Oospore progeny (N = 107) resulting from a cross between isolate OP97 (A1/IS) × SFF3 (A2/S) were subjected to AFLP analysis as described previously. Protocols for the generation, germination, and phenotypic characterization of the F1 oospores from this cross have been reported previously (9). The inheritance of AFLP bands present in one parent and absent in the other were analyzed by chi-square analysis to compare observed numbers to those expected under simple Mendelian inheritance (23). Bands present in a single parent and inherited in all the progeny were assumed to be present in two copies in the parent. Bands present in both parents, or present in two copies in one parent and absent in the other, are not reported on in this study. Individual oospore isolates were evaluated to determine if they were the products of self-fertilization or outcrossing between the parent isolates.

Clone detection. AFLP fragments for each field isolate were scored for presence or absence, and the binary data matrix was converted to a similarity matrix using a simple matching coefficient of resemblance with the program NTSYS-pc version 2.02k (Exeter Software, Setauket, NY). Unweighted pair group method with arithmetic averages (UPGMA) cluster analysis was performed on the similarity matrix and a tree was generated. Isolates showing complete homology at all loci were considered members of the same clonal lineage and, except for a single representative isolate (referred to as a clone), were excluded from population genetic analysis (13).

Population genetic analysis. Sample sets collected from single fields during a single year were considered a population. Populations were assumed to be in Hardy-Weinberg equilibrium, and each AFLP locus was assumed to be diallelic and selectively neutral. The program tools for population genetic analysis (TFPGA) (M. P. Miller, Northern Arizona University, Flagstaff) was used to assess genetic diversity within each population on the basis of estimated average heterozygosity (15) and the proportion of polymorphic loci at the 95% level (6), and to calculate pairwise and overall fixation indices (F statistics) according to the methods of Weir and Cockerham (26). Confidence intervals for F statistics at the 95% confidence level were generated by bootstrapping using 1,000 iterations.

The fixation index, as described by Wright, equals the reduction in heterozygosity expected with random mating at any one level of a population hierarchy relative to another more inclusive level of the hierarchy (27). Weir and Cockerham's approach to estimating fixation indices attempts to correct for the effects of sampling a limited number of organisms from a limited number of populations and is reported as Φ_{ST} instead of F_{ST} (26). Theoretically, the fixation index has a minimum of 0 (no loss of heterozygosity between the populations compared) and a maximum of 1 (indicating fixation for alternative alleles in different populations or a total loss of heterozygosity), but, as discussed by Hartl and Clark (6), the observed maximum is usually much less than 1. Wright (27) has suggested the following qualitative guidelines for the interpretation of fixation indices: the range 0 to 0.05 indicates little genetic differentiation, 0.05 to 0.15 indicates moderate genetic differentiation, 0.15 to 0.25 indicates great genetic differentiation, and values above 0.25 indicate very great genetic differentiation.

Using the program NTSYS-pc, the combined 0/1 data matrix for isolates from all populations was used to construct a genetic similarity matrix of all possible pairwise comparisons of individuals within and among populations using Jaccard's similarity coefficient: $GS(ij) = a/(a + b + c)$. $GS(ij)$ is the measure of genetic similarity between individuals i and j, where a is the number of polymorphic bands shared by i and j, b is the number of bands present in i and absent in j, and c is the number of bands present in j but absent in i. Trees were constructed using UPGMA cluster analysis to provide a graphic representation of the relationships among isolates. A cophenetic correlation coefficient was computed to assess the goodness of fit of the tree to the similarity matrix.

TABLE 3. Clonal component of genotypic diversity within populations of *Phytophthora capsici* from Michigan

Population[a]	Total no. of isolates	Unique AFLP genotypes (%)[b]	No. of clonal lineages	Minimum:maximum no. of isolates per clonal lineage
SW1-A98	63	57 (90)	5	2:2
SW1-A99	200	141 (71)	15	2:40
SW1-B99	71	34 (48)	12	2:9
SW1-B00	36	24 (67)	5	2:8
SC1-A98	57	50 (88)	5	2:3
SC2-B99	56	45 (80)	5	2:5
C1-A00	51	48 (94)	3	2:2
NW1-A99	88	37 (42)	12	2:12
NW2-B98	24	18 (75)	3	2:3
Total	646	454 (70)	65	...

[a] First two capital letters indicate location in Michigan with S = south, W = west, C = central, and N = north, the number following the location designator indicates the farm, the capital letter following the hyphen is a field designator, and the numbers following the field designator indicate year (e.g., 00 = 2000).

[b] Percentages calculated by dividing the number of unique amplified fragment length polymorphism (AFLP) genotypes by the total number of isolates recovered.

Genetic structure was also examined by analysis of molecular variance (AMOVA) using the ARLEQUIN software package (L. Excoffier, University of Geneva). The AMOVA analysis was used to partition the variance in banding patterns within and among populations from the same geographical site over consecutive years, between sites on the same farm separated by approximately 1 km, and between all the locations sampled in Michigan. Significance values were assigned to variance components based on a set of null distributions generated by a permutation process, which randomly assigned individuals to populations and drew 1,000 independent samples. In order to clearly summarize the spatial aspect of genetic differentiation, regression was used to fit an appropriate model to the plot of pairwise Φ_{ST} values and geographical distances.

RESULTS

AFLP band characterization and marker inheritance. Evaluation of 16 *Eco*RI + 2/*Mse*I + 2 selective primer pair combinations indicated that *Eco*RI + AC/*Mse*I + CA (EAC/MCA) provided the most clearly resolved fragment profile, and this primer pair was used to analyze DNA from the isolates in this investigation. AFLP profiles for isolate OP97, generated from separate DNA extractions on three separate occasions over a 1-year period, were identical, with only minor differences in the intensity of the electropherogram signal. Occasionally, individual reactions resulted in poorly resolved fingerprint profiles (e.g., low intensity of signal) and were repeated until signals were deemed optimal. The EAC/MCA primer combination resulted in 94 clearly resolved fragments between 40 and 550 bps when considering all the isolates recovered from Michigan.

AFLP analysis of oospore progeny from cross OP97 × SFF3 revealed that all 107 progeny had a combination of bands that were present in only a single parent, indicating that each was a product of outcrossing and not self-fertilization. A comparison of the observed to the expected ratios (1:1) for 17 bands, which were present in only one parent, indicated that only one band segregated in a manner significantly different than expected ($P = 0.05$) (Table 1). Chi-square analysis also indicated that the observed ratios of A1/A2 CT and S/IS MS were not significantly different than expected under Mendelian inheritance (Table 1).

Gene and genotypic diversity. Each isolate was scored for the presence or absence of all 94 AFLP bands. The number of AFLP bands present in each population ranged from 68 to 80, with an average of 72; the number of polymorphic bands ranged from 39 to 49, with an average of 43; and the estimated average heterozygosity ranged from 0.16 to 0.19, with an average of 0.17 (Table 2). These measurements fall within the range described for a wide range of obligately outcrossing diploid plant species. Seventeen (18%) AFLP loci were fixed for the present state (every isolate analyzed had these AFLP markers) in all populations; 12 (13%) were polymorphic in all populations, and 65 (69%) were fixed for presence or absence in some populations and polymorphic in others. The high proportion of AFLP markers fixed among the populations gives a strong indication that significant genetic differentiation exists.

Of the 646 isolates analyzed, 70% had unique multilocus AFLP profiles (Table 3). This suggests that inoculum originating from oospores plays a surprisingly large role in contributing to epidemic development. The number of clonal lineages detected from single locations in Michigan varied from 3 to 15, and the number of isolates within any single clonal lineage ranged from 2 to 40

TABLE 4. Pairwise *F* statisics (Φ_{ST})[a] (below diagonal) and geographical distances (in kilometers, above diagonal) among *Phytophthora capsici* populations in Michigan

Populations[b]	SW1-A98	SW1-A99	SW1-B99	SW1-B00	SC1-A98	SC2-B99	C1-A00	NW1-A99	NW2-B98
SW1-A98	...	0	1	1	165	169	150	180	185
SW1-A99	0.04	...	1	1	165	169	150	180	185
SW1-B99	0.18	0.25	...	0	166	170	150	180	185
SW1-B00	0.25	0.24	0.03	...	166	170	150	180	185
SC1-A98	0.36	0.37	0.29	0.29	...	8	135	260	265
SC2-B99	0.33	0.35	0.32	0.33	0.28	...	130	255	260
C1-A00	0.36	0.37	0.33	0.32	0.38	0.40	...	140	145
NW1-A99	0.32	0.34	0.30	0.30	0.32	0.32	0.38	...	5
NW2-B98	0.36	0.37	0.31	0.32	0.33	0.33	0.33	0.27	...

[a] Estimated fixation index calculated according to the methods of Weir and Cockerham.
[b] First two capital letters indicate location in Michigan with S = south, W = west, C = central, and N = north, the number following the location designator indicates the farm, the capital letter following the hyphen is a field designator, and the numbers following the field designator indicate year (e.g., 00 = 2000).

TABLE 5. Results of nested analysis of molecular variance (AMOVA) for *Phytophthora capsici* isolates based on 94 amplified fragment length polymorphism markers

Source of variation[a]	Degrees of freedom	Sum of squares	Variance component	Percent variation	P[b]
SW1-A (1998-1999)					
Among populations	1	39.658	0.396	5.05	<0.0001
Within populations	197	1,461.559	7.457	94.95	
SW1-B (1999-2000)					
Among populations	1	6.678	0.016	0.27	0.0029
Within populations	57	312.399	6.248	99.73	
SW1-A vs. SW1-B					
Among populations	1	234.790	2.762	27.34	<0.0001
Within populations	255	1,820.294	7.340	72.66	
All locations					
Among populations	6	1,169.295	4.814	39.67	<0.0001
Within populations	273	1,984.345	7.322	60.33	

[a] First two capital letters indicate location in Michigan with S = south, W = west, C = central, and N = north, the number following the location designator indicates the farm, and the capital letter following the hyphen is a field designator. Variance is partitioned between 1998 and 1999 at SW1-A, between 1999 and 2000 at SW1-B, between combined sample sets from SW1-A and SW1-B, and within and between sample sets from seven locations in Michigan. AMOVA analysis for all locations includes sample sets from a single year for locations SW1-A and SW1-B.
[b] P = the probability of obtaining a more extreme variance component estimate by chance alone based on 1,000 sampling realizations.

(Table 3). In all cases, isolates with identical multilocus AFLP profiles had identical CT and fell into the same MS category. No clones were recovered among separate locations, and cluster analysis indicated that isolates from the same location grouped discretely. The percentage of genotypically unique isolates recovered at locations ranged between 42 and 94% (Table 3). This wide variation may be due to when the samples were collected. Sample sets collected at SW1-A over the course of the 1999 growing season exhibited significantly less genotypic diversity at the end of the season due to the spread of clonal lineages (10). This is expected for an organism with polycyclic disease development and suggests that samples collected early in a *P. capsici* epidemic may provide a better estimate of genic diversity than samples collected at the height of an epidemic.

Temporal dynamics. *F* statistics (Φ_{ST}) comparing populations of *P. capsici* recovered from field SW1-A over 1998 and 1999, and field SW1-B over 1999 and 2000 were 0.04 and 0.03, respectively. These values indicate that very little genetic differentiation or loss of heterozygosity occurred between years at either location (Table 4). At both locations, the number and identity of AFLP bands resolved remained identical over time, with 72 total bands recovered from populations at SW1-A and 69 bands recovered

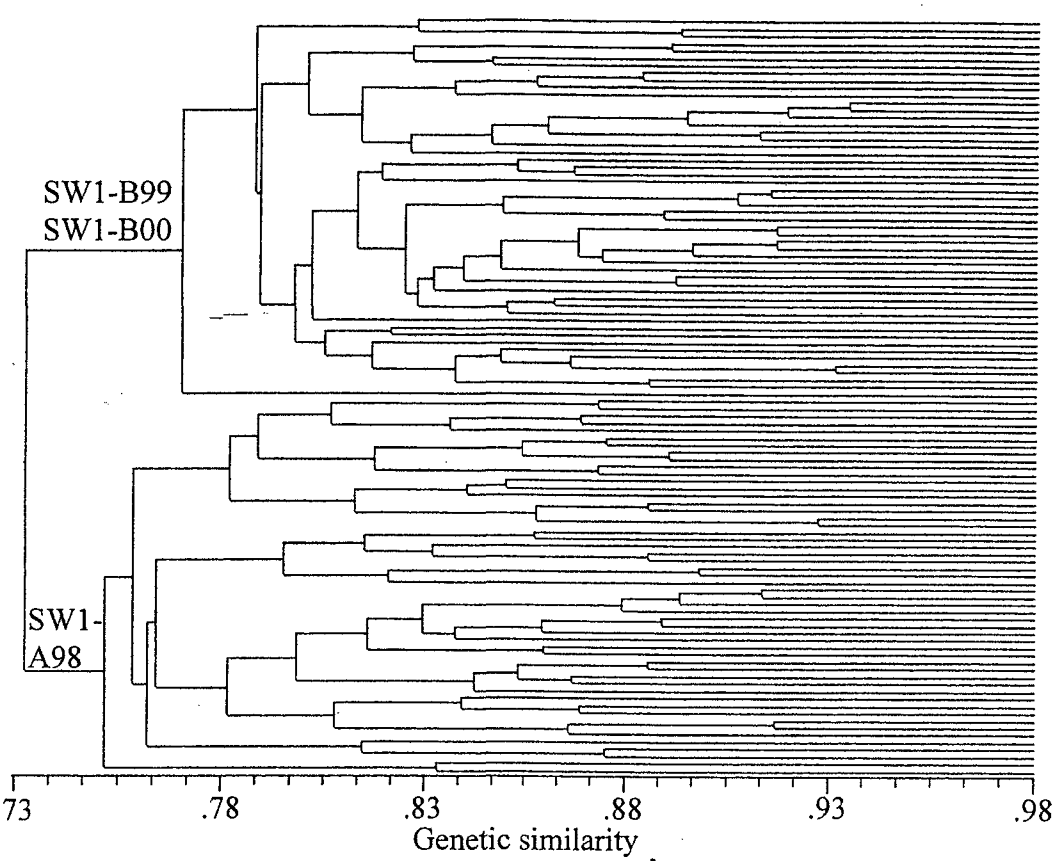

Fig. 1. Unweighted pair-group method with arithmetic average cluster analysis of *Phytophthora capsici* isolates from location SW1-B over 1999 and 2000 (N = 58) and SW1-A in 1998 (N = 57) based on the Jaccard similarity coefficient using 94 amplified fragment length polymorphism markers. Nodes contain isolates exclusively from single locations. Location identifiers precede the inclusive node and are indicated by region (S = south, N = north, W = west, and C = central) and a farm identifier (1,2,...*n*) prior to the hyphen with a field indicator (A, B,...*n*) and the year of sampling (e.g., 00 = 2000) following.

TABLE 6. Location, year, hosts, compatibility type, and mefenoxam sensitivity of genetically unique *Phytophthora capsici* isolates collected in Michigan between 1998 and 2000

			Compatibility type/mefenoxam sensitivity[d]					
Population[a]	Hosts[b]	No. of isolates[c]	A1/S	A1/IS	A1/I	A2/S	A2/IS	A2/I
SW1-A98	S, PK	57	...	4 (0.07)	31 (0.54)	...	6 (0.11)	16 (0.28)
SW1-A99	S	141	1 (0.01)	17 (0.12)	57 (0.40)	1 (0.01)	23 (0.16)	42 (0.30)
SW1-B99	S	34	14 (0.41)	4 (0.12)	...	11 (0.32)	4 (0.12)	1 (0.03)
SW1-B00	S	24	7 (0.29)	5 (0.21)	...	5 (0.21)	5 (0.21)	2 (0.08)
SC1-A98	C	50	10 (0.20)	17 (0.34)	2 (0.04)	10 (0.20)	11 (0.22)	...
SC2-B99	C	45	...	6 (0.13)	22 (0.49)	...	2 (0.04)	15 (0.33)
C1-A00	P	48	20 (0.42)	...	...	28 (0.58)	...	...
NW1-A99	S, C	37	25 (0.68)	...	...	12 (0.32)	...	...
NW2-B98	P	18	10 (0.56)	...	...	7 (0.39)	1 (0.05)	...
Total		454	87 (0.19)	53 (0.12)	112 (0.25)	74 (0.16)	52 (0.11)	76 (0.17)

[a] First two capital letters indicate location in Michigan with S = south, W = west, C = central, and N = north, the number following the location designator indicates the farm, the capital letter following the hyphen is a field designator, and the numbers following the field designator indicate year (e.g., 00 = 2000).
[b] S = squash, C = cucumber, PK = pumpkin, and P = pepper.
[c] Total number of isolates with unique multilocus amplified fragment length polymorphism profiles.
[d] Mefenoxam sensitivity determined by in vitro screening on 100 ppm of mefenoxam-amended media with S = <30% growth of control (GC), IS = between 30 and 90% GC and I = >90% GC. Observed numbers are followed by proportion of total sample size in parenthesis.

from populations at SW1-B (Table 2). The number and identity of bands polymorphic at the 95% level (37 for SW1-A and 38 for SW1-B) and the estimated average heterozygosity (0.16 for both locations) also remained constant over time (Table 2). AMOVA analysis of SW1-A and SW1-B over time partitioned 5% of the total variability between years for SW1-A and <1% of the total variability between years at SW1-B (Table 5). Significant clonal reproduction was detected at both field sites within a given year, but no members of the same clonal lineage were detected among locations or years (Table 3). Thus, even though individual genotypes did not appear to survive the winter, the data suggest that there was enough outcrossing and survival of the resulting recombinant progeny at both these locations to maintain genic diversity.

Cluster analysis showed that isolates from SW1-A and SW1-B branched from location-specific nodes (branch points on the tree). If there was migration between the locations, then isolates from SW1-A and SW1-B would be expected to be intermixed in the cluster analysis. On the other hand, there was no clustering within either of the location-specific clusters based on year (Fig. 1). The ratio of A1/A2 CT approximated a 1:1 ratio at each location (Table 6). The percentage of isolates falling into the six MS/CT categories remained relatively similar between years at each location, with a breakdown of 0 and 1% A1/S, 7 and 12% A1/IS, 54 and 40% A1/I, 0 and 1% A2/S, 11 and 16% A2/IS, and 28 and 30% A2/I for location SW1-A in 1998 and 1999, respectively (Table 6). The percentage of isolates in each of the six categories for SW1-B was 41 and 29% A1/S, 12 and 21% A1/IS, 0 and 0% A1/I, 32 and 21% A2/S, 12 and 21% A2/IS, and 3 and 8% A2/I between 1999 and 2000, respectively (Table 6).

Genetic structure. Pairwise Φ_{ST} values ranged from 0.18 to 0.40 when comparing populations from different locations (Table 4). According to Wright's criterion, this means that populations

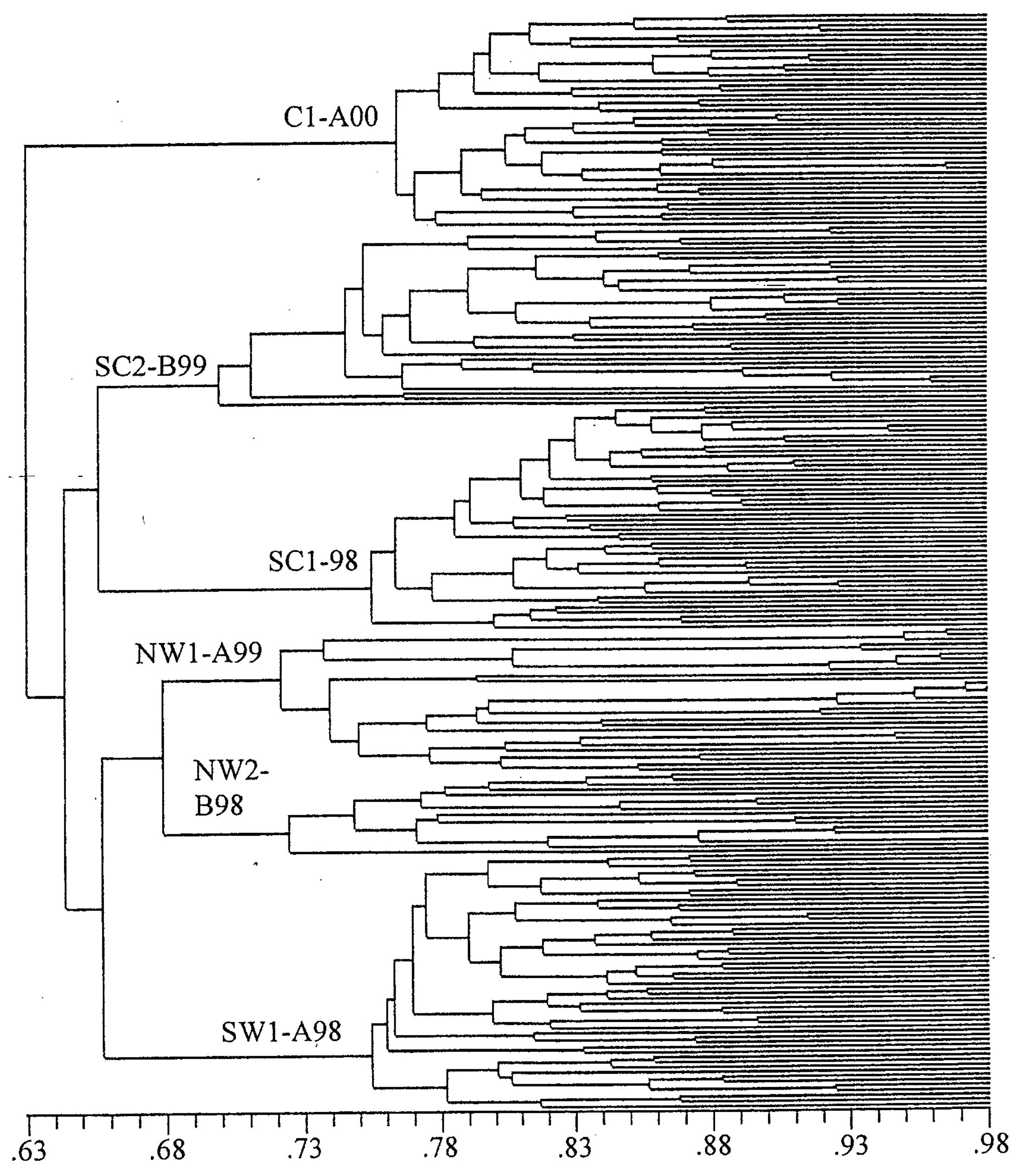

Fig. 2. Unweighted pair-group method with arithmetic average cluster analysis of 255 *Phytophthora capsici* isolates from six locations in Michigan based on the Jaccard similarity coefficient using 94 amplified fragment length polymorphism markers. Nodes contain isolates exclusively from single locations. Location identifiers precede the inclusive node and are indicated by region (S = south, N = north, W = west, and C = central) and a farm identifier (1,2,...*n*) prior to the hyphen with a field indicator (A, B,...*n*) and the year of sampling (e.g., 00 = 2000) following.

were greatly differentiated, even when located as close as 1 km apart. The overall Φ_{ST} value when analyzing isolates from all seven locations combined was 0.34 (SD = 0.03), which indicates that approximately 34% of the total genetic variation was present among locations. An AMOVA analysis of sample sets from all locations corroborated this finding and attributed 40% of the genetic variation among populations and 60% within populations (Table 5). Cluster analysis was also in agreement with the overall fixation index and revealed that populations from different geographical locations branched from specific nodes (Figs. 1 and 2), with population-specific clusters being between 63 and 75% similar. Genetic similarities between individuals within each of the clusters showed similar patterns with individuals ranging between 75 to 95% for SW1-A (1998 and 1999), 77 to 94% for SW1-B (1999 and 2000), 75 to 94% for SC1-A98, 69 to 92% for SC2-B99, 76 to 95% for C1-A00, 71 to 97% for NW1-A99, and 72 to 93% similar for NW2-B98 (Figs. 1 and 2). The cophenetic correlation coefficient for the overall tree (Fig. 2) was 0.84, indicating that the tree provided a good fit to the data matrix. The results of fitting a linear model to describe the relationship between pairwise Φ_{ST} and pairwise geographical distances indicated a significant relationship ($r^2 = 42.67$; $P < 0.01$) (Fig. 3). Although this analysis should be interpreted with caution due to the unbalanced nature of the sample (28 observations between 130 to 265 km and only 6 observations between 1 to 8 km), it suggests that the genetic differentiation among locations only becomes more substantial with increasing distance.

DISCUSSION

In Michigan, producers of tomatoes, peppers, and cucurbits have experienced increasing losses to *P. capsici* during the last 10 to 15 years. Land suitable for vegetable production is limited in some areas and the length of crop rotation is restricted. A minimum of 3-years rotation to nonsusceptible hosts is a standard recommendation (20). The efficacy of rotation in a disease management program depends on the ability of *P. capsici* to survive and move among locations. Determining the survival period of naturally produced *P. capsici* propagules is difficult (2) because inoculum may be present in a small, often undetectable amount. Although significant local spread via water has been demonstrated (21), there is little information concerning the movement of *P. capsici* among geographically separated locations. We report on isolates from seven geographically separated locations as part of an ongoing investigation aimed at determining how *P. capsici* survives and characterizing the dynamics of dispersal. Segregation analysis of 17 of the AFLP markers used in this study suggests they are generally inherited as diallelic Mendelian characters and therefore are useful for estimating population genetic measures with *P. capsici*.

Earlier studies suggest that outcrossing is an important component of the life history of *P. capsici* and that recombination has a significant impact on the genetic structure of populations (9,10). The data reported here support these previous conclusions, but suggest that outcrossing occurs on a local scale. This is best illustrated by the grouping of isolates into location-specific clusters. Gene flow among locations serves as a powerful evolutionary force to reduce genetic differentiation (6), and the distinct grouping of isolates based on location is typical for populations that are reproductively isolated. It is unlikely that incompatibility among the isolates from different locations is responsible because the progeny from the interregional cross (OP97 × SFF3) were all hybrid and previous crosses between isolates from separate populations suggested similar results (9). The estimated pairwise fixation indices and AMOVA analysis quantified the differences among the populations and indicated that >25% of the observed genetic variation was unique to single locations. Hartl and Clark state that migration of a small number of individuals (e.g., one to two) per generation is generally sufficient to keep fixation indices at 0.10 or less (6). The observed pairwise fixation indices among the populations presented here suggest that movement among locations was rare. Although polycyclic disease development appears to play an important role in disease development within a single growing season, there were no members of the same clonal lineage recovered among the seven locations or among years at SW1-A or SW1-B.

The finding that movement among locations appears to be rare suggests that the efficacy of rotation may depend more on the long-term survival of *P. capsici* than on movement among locations. The fields at farm SW1 provided a unique opportunity to investigate survival and spread. Both SW1-A and SW1-B had *P. capsici* epidemics in 1999, and the only difference among the two was previous rotation patterns. SW1-A was continuously cropped to squash from 1997 to 1999. Location SW1-B was the site of a severe *P. capsici* epidemic on squash in 1994 that was followed by a soybean and corn rotation until squash was planted again in 1999. The locations are irrigated from separate wells, do not share drainage water, and plant tissue is not knowingly moved among the sites. These fields are of particular interest because they differed significantly in the proportion of mefenoxam insensitive isolates collected in 1999. Only 2 of the 141 genetically unique isolates collected from SW1-A were sensitive to mefenoxam, whereas 24 of the 35 unique isolates recovered from SW1-B were sensitive to mefenoxam. This suggests very little, if any, movement of isolates from SW1-A to SW1-B. The patterns of diversity at the DNA level clearly separate the isolates into two discrete populations and effectively rule out gene flow in 1999. The genic stability of *P. capsici* at SW1-A from 1998 to 1999, and at SW1-B from 1999 to 2000, suggests that movement into these sites was rare. In light of these findings, a reasonable explanation for the epidemic at SW1-B in 1999 is that oospores formed during the 1994 epidemic remained dormant over five winters and provided the initial inoculum. There are reports of oospores surviving extended periods for other *Phytophthora* spp. (5), and continued tracking of the *P. capsici* populations at the locations presented here should help us decipher the relative contributions of reintroduction and survival.

The finding that population differentiation increased with distance, considering the magnitude of genetic differentiation at even the closest sites, is consistent with rare founding events originating from nearby locations or from a similar source population. For example, farms SC1 and SC2 are not connected by waterways, nor do they share equipment, but both produce cucumbers for the pickling industry and utilize the same processing station.

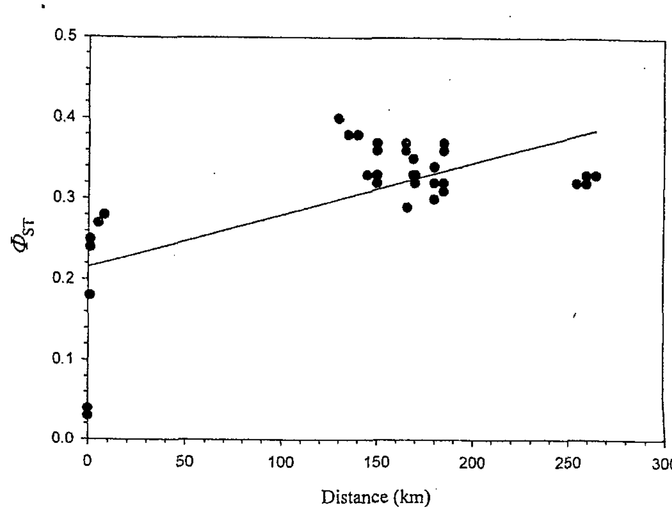

Fig. 3. Association between the degree of genetic subdivision (pairwise Φ_{ST}) and pairwise geographical distances among *Phytophthora capsici* populations at seven locations in Michigan ($r^2 = 42.6$; $P < 0.01$).

When a semitrailer of cucumber fruit is delivered to the processing station the weight of oversized, undersized, or diseased cucumbers (culls) is estimated and the trailer is reloaded with a corresponding weight of culls sorted from previous deliveries. Traditionally, these culls are spread onto fields with a manure spreader. A single cucumber cull infected with A1 and A2 CT may contain thousands of oospores (K. H. Lamour and M. K. Hausbeck, *unpublished data*) and it is possible that transfer of infected culls may have contributed to the dissemination of *P. capsici* in Michigan. All of the locations sampled in this study had a history of *P. capsici* epidemics and investigation of a newly infested field should provide insight into the nature of founding events.

In summary, it appears that *P. capsici* persists in Michigan fields as reproductively isolated outcrossing populations in which the sexual stage is effectively linked to long-term survival. Thus, even though single genotypes have the potential to increase significantly within a single season, genic diversity is maintained over time and new gene combinations are constantly generated. Comparison of future sample sets to the baseline data presented here should provide an opportunity to further clarify the contributions of movement among locations and survival to the population structure of *P. capsici* in Michigan.

ACKNOWLEDGMENTS

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Worksheet 3-A(17)(c). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. <u>You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative</u>. Use additional pages as need.

<u>For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed</u>. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: <u>Crop Rotation, Fallow</u> **Study: <u>The dynamics of mefenoxam insensitivity in a recombining population of *Phytophthora capsici* characterized with amplified fragment length polymorphism markers.</u>**

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted ___X___
1b. Township caps ______
1c. Alternative not acceptable in consuming country ______
1d. Other (Please describe) ______

____
____
____
____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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Worksheet 3-A(17)(c). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. **Is the study on EPA's website?** **Yes** ______ **No** X

   1a. **If not on the EPA website, please attach a copy.**

2. **Author(s) or researcher(s)** K.H. Lamour
   M.K. Hausbeck

3. **Publication and Date of Publication** Phytopathology 91:553-557, 2001

4. **Location of research study** Michigan

5. **Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.**
   Crop Rotation

6. **Was crop yield measured in the study?** **Yes** ______ **No** X

7. **Describe the effectiveness of the alternative in controlling pests in the study.**
   Oospores of the soil-borne fungus play a key role in overwintering and the frequency of mefenoxam insensitivity may not decrease in an agriculturally significant time period (2 years), rendering crop rotation and fungicide use ineffective.

8. **Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?**
   This study is directly applicable, since it was conducted in Michigan and documents the situation of commercial farms.

The Dynamics of Mefenoxam Insensitivity in a Recombining Population of *Phytophthora capsici* Characterized with Amplified Fragment Length Polymorphism Markers

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ABSTRACT

Lamour, K. H., and Hausbeck, M. K. 2001. The dynamics of mefenoxam insensitivity in a recombining population of *Phytophthora capsici* characterized with amplified fragment length polymorphism markers. Phytopathology 91:553-557.

Recent findings from Michigan suggest that recombination may play a role in the survival and evolution of sensitivity to the fungicide mefenoxam in populations of *Phytophthora capsici* on cucurbit hosts. In 1998, 63 mefenoxam insensitive isolates were recovered from a squash field in which mefenoxam had been applied. Additional isolates were recovered from untreated squash fields planted at this location in 1999 (200 isolates) and the spring of 2000 (34 isolates). Isolates from 1998 and 1999 were characterized using fluorescent amplified fragment length polymorphism (AFLP) markers and all isolates were screened for compatibility type and mefenoxam sensitivity. In 1998 and 1999, 92 and 71% of the isolates, respectively, had unique multilocus AFLP genotypes with no identical isolates recovered between years. Seventy-two identical AFLP markers were clearly resolved in both the 1998 and 1999 sample sets, and fixation indices for the 37 polymorphic AFLP loci indicate little differentiation between years. There was no decrease in the frequency of resistant isolates during the 2 years without mefenoxam selection. We conclude that oospores play a key role in overwintering and that the frequency of mefenoxam insensitivity may not decrease in an agriculturally significant time period (2 years) once mefenoxam selection pressure is removed.

Additional keywords: fungicide resistance, genetic diversity, population genetics.

Crown, root, and fruit rot caused by *Phytophthora capsici* is increasing in Michigan cucurbit production fields, and uninfested land suitable for rotation is becoming increasingly scarce, especially in areas undergoing rapid urban development. The phenylamide fungicide (PAF) mefenoxam is a systemic fungicide that appears to be acting at the level of DNA translation, and is fungistatic to fully sensitive isolates of *P. capsici* (2,13). Although mefenoxam has been considered by some growers to be helpful, mefenoxam insensitive isolates were reported on bell peppers in North Carolina and New Jersey by Parra and Ristaino in 1998 (18) and have since been recovered from 10 of 11 farms sampled in Michigan (13), as well as, in Georgia (15) and southern Italy (19). Mefenoxam insensitivity in Michigan *P. capsici* isolates is inherited as a single gene exhibiting incomplete dominance (13), which is consistent with the reports for a variety of other oomycetous organisms (2). Investigations with *P. infestans* indicate that insensitivity may be conferred by genes at different chromosomal positions (5), suggesting that the basis of insensitivity in different populations may not be identical. Sexual recombination, in particular, has the potential to impact management strategies that employ PAFs because the fully insensitive (two copies of the insensitivity allele) phenotype may be directly generated. *P. capsici* is heterothallic and the sexual stage is initiated when isolates of opposite compatibility type, designated A1 and A2, come into close association to form thick-walled oospores (4). The asexual stage includes the production of caducous sporangia born on long pedicels, which may release motile zoospores if free water is present. Asexual spores are thought to be responsible for the polycyclic nature of disease development (20).

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PAF resistance in the genus *Phytophthora* and, in particular, the *P. infestans*–potato pathosystem, is well documented (2,4,9). Until recently, the population structure of *P. infestans* appeared to be largely clonal outside of *P. infestans* putative center of origin (6). The recent detection of both *P. infestans* compatibility types along with increased genotypic diversity in some potato growing regions indicates that the sexual stage is likely active and may significantly impact control strategies that have proved useful in the past (3,8). When PAF resistance in European *P. infestans* populations increased significantly in the early 1980s, the efficacy of the PAF metalaxyl was only regained after the product was not made available to growers for a period of time (2). This strategy apparently allowed the resistant populations to decline or become extinct and depends on ephemeral populations or, in the case of resident populations, upon a significant cost for resistance outside of selection pressure. A recent study of sensitive versus PAF resistant *P. nicotianae* isolates from citrus suggests negligible fitness costs for PAF resistance and reports that 2 years without PAF use did not reduce the proportion of resistant isolates in groves (21). Kadish and Cohen report that PAF-resistant *P. infestans* isolates in Israel were more aggressive in colonizing tuber tissue than sensitive isolates (12).

Novel techniques have been developed recently that allow characterization of DNA-level polymorphism in organisms for which little is known about the genome. An example is the amplified fragment length polymorphism (AFLP) technique introduced by Vos et al. in 1995 (23). This technique relies on restriction enzyme fragmentation of genomic DNA with the concomitant ligation of synthetic adaptors to the DNA fragment ends. Stringent polymerase chain reaction (PCR) amplification using adaptor-complementary primers with additional selective nucleotides allow for the amplification of fragment subsets. DNA fragment subsets are termed fingerprints and may be resolved with a range of techniques (1). AFLP markers have been used on a variety of organisms (14,22) and the procedure generates a large number of reproducible markers (1,22). The limitation that these markers are generally scored as dominant markers (e.g., either present or absent) for diploid organisms requires the use of relatively large sample sets (11,25).

Our null hypotheses are that sexual recombination has a significant impact on the population structure of *P. capsici* in Michigan and that mefenoxam insensitivity may not decrease in the time frame of a typical 2-year rotation outside of mefenoxam selection pressure.

MATERIALS AND METHODS

Field plot. Research was conducted on a commercial farm in southwest Michigan, with a history (>11 years) of *P. capsici* on bell peppers and squash and intensive use of PAF. The 4.05-ha field sampled had previously been cropped to soybeans and corn with no known record of *P. capsici* susceptible crops (e.g., tomatoes, peppers, or cucurbits) prior to 1997. During 1997 and 1998, yellow squash and zucchini grown in this field became diseased with *Phytophthora* crown, root, and fruit rot and the grower applied mefenoxam as part of a disease management strategy (Novartis, Greensboro, NC). In 1998, all isolates recovered were either intermediately or fully insensitive to mefenoxam. Both A1 and A2 compatibility types were present, and oospores were detected in diseased fruit. In 1999 and 2000, yellow squash was established in a 1,124-m^2 experimental plot in this field, and mefenoxam was not applied. Diseased plants and fruit were sampled on 20 August 1998 (63 isolates from entire field), June through August 1999 (200 isolates from experimental plot), and 13 July 2000 (34 isolates from experimental plot). All isolates were recovered from single diseased plants or fruit.

TABLE 1. Fixation indices (F_{ST}) for 37 amplified fragment length polymorphism loci from unique *Phytophthora capsici* isolates collected from a single Michigan cucurbit field during 1998 ($N = 57$) and 1999 ($N = 141$)

Fragment[a]	1998 f(aa)[b]	1999 f(aa)	F_{ST}[c]
45	0.02	0.06	0.018
54	0.29	0.29	0.000
64	0.82	0.55	0.048
104	0.11	0.06	0.007
106	0.11	0.04	0.025
110	0.41	0.36	0.002
130	0.41	0.30	0.009
146	0.47	0.24	0.038
149	0.12	0.27	0.029
154	0.39	0.31	0.004
156	0.53	0.83	0.054
172	0.56	0.33	0.034
189	0.16	0.56	0.121
192	0.16	0.37	0.044
193	0.35	0.20	0.022
211	0.47	0.15	0.088
241	0.48	0.32	0.018
256	0.04	0.01	0.022
258	0.43	0.49	0.002
261	0.55	0.54	0.000
270	0.57	0.41	0.015
282	0.35	0.40	0.002
285	0.51	0.73	0.030
314	0.51	0.34	0.019
320	0.41	0.51	0.006
333	0.16	0.20	0.002
346	0.36	0.33	0.001
361	0.33	0.49	0.017
383	0.21	0.15	0.005
418	0.40	0.34	0.002
431	0.34	0.32	0.001
438	0.67	0.45	0.028
454	0.65	0.49	0.015
492	0.29	0.40	0.009
504	0.51	0.47	0.001
511	0.38	0.28	0.007
548	0.78	0.78	0.000

[a] *Eco*RI-AC/*Mse*I-CA selectively amplified fragment size in base pairs.

[b] Observed frequency of the absent state where "a" represents the absence of a fragment.

[c] F_{ST} calculated from estimated allele frequencies. According to Wright's qualitative guidelines, values from 0 to 0.05 indicate little genetic differentiation and values from 0.05 to 0.15 indicate moderate genetic differentiation.

Isolate collection and maintenance. Isolation from diseased plant material was made onto BARP (25 ppm of benomyl, 100 ppm of ampicillin, 30 ppm of rifampicin, and 100 ppm of pentachloronitrobenzene)-amended UCV8 (840 ml of distilled water, 163 ml of unclarified V8 juice, 3 g of $CaCO_3$, and 16 g of Bacto agar) plates. Procedures for obtaining single zoospore isolates were as previously described (13). Single zoospore cultures were maintained on 30 ppm of rifampicin and 100 ppm of ampicillin (RA)-UCV8 plates and transferred bimonthly. Long-term storage consisted of a single 7-mm plug of expanding mycelium from each single zoospore culture being placed in a 1.5-ml microfuge tube with one sterilized hemp seed and 1 ml of sterile distilled water, incubated for 2 to 3 weeks at 23 to 25°C, and stored at 15°C long term.

Phenotypic characterization. Isolates were screened for compatibility type as previously described (13). Mefenoxam sensitivity was characterized according to the in vitro screening technique described by Lamour and Hausbeck (LH technique) for *P. capsici* isolates in Michigan (13). Isolates were scored as sensitive (S) if growth on UC-V8 agar amended with 100 ppm of mefenoxam was less than 30% compared with a control, as intermediately sensitive (IS) if between 30 and 90%, and fully insensitive (I) if greater than 90% compared with the unamended control. These mefenoxam sensitivity categories are based on a trimodal distribution of 523 field isolates of *P. capsici*. Clear modal distributions were only attained when screening was conducted with a single high rate of mefenoxam-amended (100 ppm) media (K. Lamour, *unpublished data*). These putative mefenoxam sensitivity categories were tested by in vitro crosses (I × S, IS × IS, IS × S, and S × S), and chi-square analysis confirmed that the observed progeny numbers were not significantly different than expected for Mendelian inheritance of an incompletely dominant trait (13).

The LH technique differs from a commonly used method described by Goodwin, Sujkowski, and Fry (GSF technique) (9) for *P. infestans* which uses two levels of amended media (5 and 100 ppm) to differentiate the three mefenoxam sensitivity phenotypes and which has been used to characterize *P. capsici* isolates (15,18,19). Unfortunately, analysis of our in vitro crosses and field isolates by the GSF technique did not resolve a clear modal distribution (K. Lamour, *unpublished data*). Assignment of Michigan *P. capsici* isolates to the S category was the same whether using the LH or GSF technique. The only difference was that some *P. capsici* isolates from Michigan rated as fully insensitive by the GSF technique were rated as intermediately sensitive by the LH technique.

DNA extraction and AFLP fingerprinting. A technique for avoiding bacterial contamination prior to growing isolates for DNA extraction was implemented using a modified Van Teigham cell (4). The uppermost portion of a 7-mm plug of mycelium was placed onto the surface of RA-WA plates (30 ppm of rifampicin, 100 ppm of ampicillin, 1,000 ml of distilled water, and 16 g of Bacto agar) and an autoclaved cap from a 1.5-ml microfuge tube was placed over the plug which forced the isolate to grow through the amended media. Isolates were incubated in the dark for 2 to 3 days before two 7-mm plugs were transferred to approximately 15 ml of RA-UCV8 broth in petri dishes (100 × 15 mm) and incubated in the dark for 3 days at 23 to 25°C. Mycelial mats were washed with distilled water and dried briefly under vacuum before being frozen to –20°C and lyophilized.

Lyophilized mats were ground with a sterile mortar and pestle. Whole genomic DNA from approximately 50 mg of ground mycelium was extracted with a plant mini kit (Dneasy; Qiagen Inc., Valencia, CA) according to the manufacturers directions. DNA was quantified (Nucleic Acid QuickSticks; Clontech, Palo Alto, CA) according to the manufacturers directions and approximately 100 ng of DNA was subjected to a restriction/ligation reaction, preselective amplification, and selective amplifications using the PCR core mix, adaptor sequences, core primer sequences, and fluorescent-labeled primers available in an AFLP microbial fingerprinting kit (Perkin-Elmer Applied Biosystems, Foster City, CA, henceforth referred to as PE/ABI) and performed exactly as described in protocol part 402977 Rev A (23). All PCR reactions were performed with a minicycler (MJ Research Inc., Waltham, MA) in 0.2-ml tubes according to the cycling parameters outlined in the microbial fingerprinting protocol.

An initial optimization set of reactions was performed with preselective products from *P. capsici* isolate OP97, which was isolated from a cucumber fruit in 1997 (13). Selective amplifications with the selective primers *Eco*RI-AA, -AC, -AG, and -AT were performed in all 16 combinations with the *Mse*I-CA, -CC, -CG, and -CT selective primers. *Eco*RI selective primers, available from PE/ABI, were labeled at the 5′ end with either carboxyfluorescein (FAM), carboxytetramethyrhodamine (TAMRA), or carboxy-4′,5′-dichloro-2′,7′-dimethoxyfluorescein (JOE) fluorescent dyes. The fluorescent dyes are excited by laser radiation and visualized by their characteristic absorption-emission frequencies. Only the fragments containing an *Eco*RI restriction site are resolved.

Products from three reactions labeled with different colored dyes and a carboxy-X-rhodamine (ROX) size standard were loaded into each lane on a denaturing polyacrylamide gel and the fragments resolved in a DNA sequencer (ABI Prism 377). Results were prepared for analysis in the form of electropherograms using GeneScan Analysis software (PE/ABI). AFLP fragments were scored manually as present (1) or absent (0) using Genotyper (PE/ABI). Only DNA bands that consistently exhibited unambiguous presence or absence profiles were scored.

A single isolate, OP97, was subjected to the aforementioned protocol using three primer pair combinations that were chosen as optimal on three separate occasions, approximately 3 months apart, to test for reproducibility of AFLP profiles.

Clone detection and cluster analysis. AFLP fragments were considered polymorphic if the most common allele was present in less than 95% of the isolates from a given sample set and scored for presence (1) or absence (0) (10). AFLP fragments present in more than 95% of the isolates from a given sample set were considered monomorphic. Analysis of the resulting binary data matrix was performed using NTSYS-pc version 2.02k (Exeter Software, Setauket, NY). Unweighted pair group method with arithmetic averages cluster analysis was performed on the matrix of similarity coefficients calculated from all possible pairwise comparisons of individuals within and among the 1998 and 1999 populations and a tree generated. Isolates showing complete homology at all loci were considered to be clones and except for a single representative isolate were excluded from frequency calculations.

Allele frequency and fixation indices. Allele frequencies for AFLP markers were estimated utilizing the expected relationship between gene and genotype frequencies in a randomly mating population (i.e., Hardy-Weinberg proportions). The frequency of the recessive (absent) allele (q) was calculated from the observed number of recessive homozygote individuals (X) in a sample of n individuals by the formula for dominant markers described by Jorde et al. (11):

$$\hat{q} = \sqrt{x + \frac{1-x}{4n}}$$

where $x = X/n$ is the observed proportion of individuals that do not display the dominant (present) marker phenotype. In order to test whether the composite genetic profiles from 1998 and 1999 were consistent with a single randomly mating population, the fixation index was calculated for each AFLP loci from the variance in allele frequencies according to the following formula: $F_{ST} = [(p_1-p_2)^2/4]/(\text{average } p \times \text{average } q)$, where p is the allele frequency for the present state with p_1 and p_2 indicating the two sample populations, and q is the allele frequency for the absent state (10). Fixation indices for individual loci were interpreted according to the qualitative guidelines suggested by Wright (24), where the range 0 to 0.05 indicates little genetic differentiation, range 0.05 to 0.15 indicates moderate genetic differentiation, and greater than 0.25 indicates great genetic differentiation (10).

RESULTS

AFLP band characterization. Evaluation of the 16 *Eco*RI + 2–*Mse*I + 2 selective primer pair combinations indicated that *Eco*RI + AC–*Mse*I + CA gave the most clearly resolved fragment profile and was used to amplify genomic DNA from all isolates in both the 1998 and 1999 sample sets. This primer combination resulted in 72 clearly resolved fragments of which 37 (51%) fragments were polymorphic in both 1998 and 1999 (Table 1). All 72 fragments were present in both 1998 and 1999 and no novel fragments were detected between years. The following 35 fragments (size in base pairs) were monomorphic in both the 1998 and 1999 sample sets: 41, 43, 47, 49, 58, 66, 70, 82, 85, 114, 118, 123, 133, 135, 140, 159, 174, 235, 247, 249, 272, 278, 295, 298, 300, 341, 351, 355, 367, 402, 474, 488, 502, 519, and 527. AFLP profiles for isolate OP97, generated from separate DNA extractions on three separate occasions over a 1-year period, resulted in identical banding patterns with the only difference being minor changes in the intensity of the electropherogram signal. Occasionally individual reactions resulted in poorly resolved fingerprint profiles (e.g., low intensity of signal) and were repeated until signals were deemed optimal.

Phenotypic, genotypic, and gene diversity. No isolates sensitive to mefenoxam were recovered in 1998 or 2000, and single A1 sensitive and A2 sensitive isolates were recovered in 1999 (Table 2). In 1998, 18% of the isolates were intermediately sensitive and 82% were insensitive, in 1999, 2% were sensitive, 28% were intermediately sensitive and 70% were insensitive, and in 2000, 15% of the isolates were intermediately sensitive and 85% were insensitive to mefenoxam (Table 2).

In 1998, 57 of the 63 isolates recovered, and 141 of the 200 isolates recovered in 1999 were unique based on multilocus AFLP profiles. No identical multilocus genotypes were recovered between 1998 and 1999. Five isolates (two A2/I, two A2/IS, and

TABLE 2. Phenotypic diversity of *Phytophthora capsici* isolates recovered from the same cucurbit field in 1998, 1999, and 2000

Year[a]	No. of isolates[b]	Compatibility type and mefenoxam sensitivity[c]					
		A1/S	A1/IS	A1/I	A2/S	A2/IS	A2/I
1998	57	–	4	31	–	6	16
1999	141	1 (2)	17 (20)	57 (53)	1 (1)	23 (18)	42 (47)
2000	34	–	2	8	–	3	21

[a] Mefenoxam was applied in 1998 but not in 1999 or 2000.

[b] Sample sets from 1998 and 1999 consist of unique multilocus genotypes as determined with amplified fragment length polymorphism fingerprinting. The 2000 sample set was recovered at the beginning of the growing season and was not fingerprinted.

[c] S = sensitive, IS = intermediately sensitive, and I = insensitive as determined by in vitro screening on 100 ppm of mefenoxam-amended agar. Numbers in parentheses indicate the expected number of isolates when mefenoxam insensitivity is assumed to be controlled by a single incompletely dominant gene in Hardy-Weinberg equilibrium unlinked to compatibility type.

one A1/I) of *P. capsici* collected in 1998 had one clonal representative. Fourteen isolates collected in 1999 had between two and four clones (Table 3). A single A1 compatibility type insensitive isolate had 40 clones recovered over the course of the 1999 season and comprised 3% of the early, 15% of the mid-, and 43% of the late sampling intervals (Table 3). The 1999 sampling intervals (early, mid, and late) are based on the dates of sampling and are not intended to reflect stages of plant growth or the epidemiology of *P. capsici*. Cluster analysis of AFLP fingerprint variation indicated no significant clustering of isolates between 1998 and 1999.

The majority (98%) of the 37 polymorphic AFLP fragments showed little genetic differentiation ($F_{ST} < 0.05$) between 1998 and 1999 according to Wrights qualitative criterion (Table 1) (24).

DISCUSSION

P. capsici causes significant damage to cucurbit hosts in Michigan each year. In an effort to prevent or control epidemics, many growers have used either metalaxyl or the newer, but similarly acting compound, mefenoxam as a part of their disease management strategy. This study was initiated in an effort to address the concerns of growers who have high levels of mefenoxam insensitivity.

Phenotypic data (mefenoxam sensitivity and compatibility type) from a 1998 survey suggested that insensitivity to mefenoxam was common and that some level of recombination is occurring in the field (13), but without the application of additional polymorphic markers our ability to assess population structure was severely restricted. AFLP analysis proved to be a powerful tool for resolving the population dynamics of *P. capsici*. A single selective primer combination, *Eco*RI-AC–*Mse*I-CA, generated 72 bands of which 37 were polymorphic in our 1998 and 1999 sample sets. AFLP fingerprinting, in conjunction with temporal sampling, provided a useful characterization of *P. capsici* from one season to the next and allowed us to track asexual disease development over the course of a single season.

Our data suggests that sexual recombination significantly impacts the structure of this *P. capsici* population. The finding that 198 of the 262 isolates recovered between 1998 and 1999 had unique multilocus AFLP genotypes is consistent with the high level of genotypic diversity expected in an outcrossing population (7,16,17). Although clonal reproduction occurred in 1998 and 1999, no identical genotypes were recovered between years, suggesting that oospores are important for overwintering. The finding that 35 of the 37 polymorphic fragments exhibited very little differentiation (i.e., change in allele frequency) based on the estimated fixation indices between 1998 and 1999 is consistent with the expectations for a recombining population large enough to avoid dramatic changes due to genetic drift.

In 1999 and 2000, sensitive and intermediately sensitive isolates (42 of 175) did not increase in a manner suggesting selection in favor of mefenoxam sensitivity outside of mefenoxam selection pressure. The fact that 14 of the 15 isolates with clonal reproduction in 1999 were fully insensitive may be another indication that mefenoxam insensitivity does not have significant costs outside of mefenoxam selection pressure. If we assume that there is only a single mefenoxam insensitivity gene in this population unlinked to compatibility type, designated *I*, and that this population is effectively free from the effects of migration and genetic drift, some interesting speculations can be made. For instance, in 1999, if the mefenoxam sensitivity phenotypes are assumed to represent genotypes (e.g., a fully insensitive isolate has two copies of the *I* allele) then the frequency of *I* can be estimated and the observed number of unique isolates that fall into each of the six mefenoxam sensitivity/compatibility type categories can be compared with the expectations under Hardy-Weinberg equilibrium. In 1999, the estimated frequency of *I* was 0.84, and chi-square analysis, using the data in Table 2, indicates that the observed numbers do not differ from those expected under Hardy-Weinberg equilibria at $P = 0.50$ ($\chi^2 = 3.09$, df = 4). Although this is not a particularly powerful test due to the large number of assumptions (10), it does lend support to the hypothesis that this population meets the criterion for panmixia.

Our results do not allow us to reject the null hypothesis that sexual recombination significantly impacts the structure of this population. It appears that sexual recombination plays a significant role in maintaining genotypic and gene diversity while concomitantly producing overwintering inoculum. Our data also suggest that sexual recombination may serve as a potent force for integrating a beneficial allele based on the finding that there were a total of 133 unique multilocus genotypes fully insensitive to mefenoxam between 1998 and 1999. An interesting question that can only be answered by following a fully sensitive population as it shifts to insensitivity is how much genetic diversity is lost, if any, during the PAF selection process? The question of how long mefenoxam resistance will remain in a population of *P. capsici* when selection pressure is removed can only be answered in a tentative way. It appears that in this population, insensitivity will not decrease within the time frame of a typical 2-year rotation and, once resistance to mefenoxam is established, the future usefulness of this fungicide may be extremely limited.

Comparison of the population structure reported at this single location is currently being compared with other locations in Michigan and the United States and should provide useful insight into the amount of genetic diversity in sensitive versus insensitive populations as well as the contribution of migration to *P. capsici* population structure.

TABLE 3. Clone contribution of 15 *Phytophthora capsici* isolates to the total number of isolates collected in 1999 ($N = 200$)

Isolate	No. of clones[a]	CT/MS[b]	No. of clones in early, mid, and late season[c] 6/22 – 7/16 $N = 60$	7/20 – 8/3 $N = 80$	8/5 – 8/18 $N = 60$
JP571	2	A1/I	2	–	–
JP583	2	A1/I	2	–	–
JP944	3	A1/I	2	1	–
JP999	3	A1/I	2	1	–
JP1007	2	A1/I	1	1	–
JP1042	2	A2/I	1	1	–
JP1096	2	A1/I	–	1	1
JP1102	2	A2/I	–	2	–
JP1215	3	A2/I	3	–	–
JP1342	2	A2/IS	–	2	–
JP1369	2	A1/I	1	1	–
JP1384	4	A2/I	3	1	–
JP1512	2	A1/I	1	–	1
JP1555	3	A1/I	–	–	3
JP1632	40	A1/I	2	12	26

[a] Total number of isolates with identical multilocus amplified fragment length polymorphism profiles.
[b] CT = compatibility type and MS = mefenoxam sensitivity where S = sensitive, IS = intermediately sensitive, and I = insensitive as determined by in vitro screening on 100 ppm of mefenoxam-amended agar.
[c] Sample intervals based on sampling dates only.

ACKNOWLEDGMENTS

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Worksheet 3-A(17)(d). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. <u>You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative</u>. Use additional pages as need.

<u>For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed</u>. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: <u>Crop Rotation</u> **Study: <u>The spatiotemporal genetic structure of *Phytophthora capsici* in Michigan and implications for disease management.</u>**

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted __X__
1b. Township caps ______
1c. Alternative not acceptable in consuming country ______
1d. Other (Please describe) ______

____

____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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Worksheet 3-A(17)(d). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. **Is the study on EPA's website?** **Yes** ______ **No** X

   1a. If not on the EPA website, please attach a copy.

2. **Author(s) or researcher(s)** K.H. Lamour
   M.K. Hausbeck

3. **Publication and Date of Publication** Phytopathology 92:681-684, 2002

4. **Location of research study** Michigan, USA

5. **Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.**
   Crop Rotation

6. **Was crop yield measured in the study?** **Yes** ______ **No** X

7. **Describe the effectiveness of the alternative in controlling pests in the study.**
   Crop rotation is not highly effective because both mating types of *Phytophthora capsici* are present in Michigan fields, resulting in an oospore capable of surviving for long periods of time.

8. **Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?**
   The results of this study are directly applicable since the research was conducted in Michigan, USA.

The Spatiotemporal Genetic Structure of *Phytophthora capsici* in Michigan and Implications for Disease Management

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Root, crown, and fruit rot caused by *Phytophthora capsici* Leonian is a limiting factor for the production of peppers, tomatoes, and cucurbit crops in Michigan and the United States. Like many species in the genus *Phytophthora*, *P. capsici* has the potential for rapid polycyclic disease development from a limited amount of initial inoculum (6). *P. capsici* produces caducous sporangia that can be spread by wind-blown rain or release 20 to 40 motile zoospores in the presence of free water. The polycyclic phase of disease development is thought to be driven primarily by asexual spore dispersal at a local scale (within and down rows). Sexual reproduction requires both the A1 and A2 compatibility types (CTs) and results in the production of thick-walled oospores. Oospores are thought to serve as the primary survival structure outside of host tissue.

Recommended disease management strategies stress the importance of avoiding excess water in the plant rhizosphere by using well-drained fields, conservative irrigation, and planting on raised beds. Additional recommendations include rotation to nonsusceptible hosts for at least 2 years and the use of fungicides. The phenylamide fungicide (PAF) mefenoxam is a systemic compound with high activity against *P. capsici* and has been used by growers throughout the United States to control *P. capsici*. Insensitivity to PAF has been reported for a number of other oomycetous organisms (*Bremia lactucae*, *P. infestans*, and *P. sojae*, etc.) and appears to be conferred by a single incompletely dominant gene of major effect (1). Growers in Michigan practicing 2+-year rotation in well-drained fields using an array of fungicidal management tools have experienced significant losses to *P. capsici*. Michigan is the number one producer of cucumbers for pickling in the United States and it was at the request of grower groups associated with this industry that research into the epidemiology and reproductive biology of *P. capsici* on cucurbit hosts was initiated.

Although many researchers cite oospores as the most likely propagule for survival outside of host tissue, there have been very few investigations specifically aimed at determining the impact of sexual reproduction in natural populations of *P. capsici*. Our hypothesis was that the sexual stage may play an important role not only in survival but also in the adaptation of *P. capsici* populations to environmental stresses (e.g., fungicides). Our goal was to perform a comprehensive investigation of the phenotypic and genetic diversity present in *P. capsici* populations from the major vegetable production regions of Michigan, with the implicit intention of addressing questions concerning epidemiology, reproductive biology, and the durability of currently recommended management strategies.

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METHODOLOGY

Isolate collection and maintenance. Sampling of diseased fields began at the end of the 1997 growing season and continued through September 2000. In all cases, fields were sampled on a grid with quadrants varying from 40 m^2 to 12 km^2. A limited number of isolates were collected in 1997. In 1998, the strategy was to collect as many samples from as many fields as possible. This strategy was modified in 1999 and 2000 to focus on specific fields. Isolations from diseased plants were made onto selective media and single zoospore cultures were generated according to standard single sporing techniques (3). Isolates were placed into long-term storage (15°C) using a hemp seed/sterile water technique.

Phenotypic characterization. Single zoospore isolates were screened for CT using known A1 and A2 isolates. In vitro screening techniques published for other *Phytophthora* species for assessing sensitivity to mefenoxam were compared and a novel, simple, high dose screen using 100 ppm of mefenoxam-amended V8 agar was found to separate field isolates into three modal distributions that appeared consistent with the expectations of a single incompletely dominant gene governing mefenoxam insensitivity (e.g., sensitive, intermediately sensitive, and fully insensitive). These putative mefenoxam sensitivity (MS) groupings were tested by performing a series of crosses and testing whether the observed progeny sets met the expectations for Mendelian inheritance of a single incompletely dominant gene controlling insensitivity to mefenoxam. Sexual crosses were conducted on unclarified V8 agar plates and incubated for 3 months in the dark. Individual germinated oospores were recovered after 3 months using previously published techniques (2).

The efficacy of this in vitro mefenoxam screening technique was further tested in pumpkin seedlings using progeny from a cross between parents intermediately sensitive to mefenoxam. Nine isolates from each of the three MS categories were screened for pathogenicity on untreated seedlings. Single sensitive, intermediately sensitive, and fully insensitive isolates were then placed onto the unwounded surface of plants treated with either a field rate of mefenoxam, three times the field rate, or distilled water. Lesion diameters on seedling stems were measured after 4 days.

Genetic characterization. Single zoospore isolates were grown in antibiotic-amended V8 broth for 3 days at room temperature. Mycelial mats were washed, frozen, lyophilized, and ground with a sterile mortar and pestle. DNA was extracted with either a Qiagen Dneasy extraction kit (Qiagen, Valencia, CA) or via a cetyltrimethylammonium bromide (CTAB) procedure. A variety

of methods for generating molecular markers were tested for efficacy including isozyme, random amplified polymorphic DNA, and amplified fragment length polymorphism (AFLP). The AFLP technique resulted in a large number of reproducible markers and was chosen to characterize samples of *P. capsici* from Michigan. The AFLP technique involves cutting genomic DNA with moderately rare cutting (*Eco*RI) and frequent cutting (*Mse*I) restriction enzymes, while concomitantly ligating synthetic adaptor fragments of DNA to the sticky ends created by the restriction enzymes (7). The result is a large number of DNA fragments that have ends with known DNA sequences. Amplification of fragment subsets (termed fingerprints) can be accomplished using polymerase chain reaction (PCR) primers complementary to the adaptor sequences with additional "selective" nucleotides. Changing the amount and type of selective nucleotides results in different subsets or fingerprints. Stringent PCR cycling parameters (touchdown technique) are used to ensure the fidelity of the reaction. For the analysis summarized here, adaptor sequences and fluorescent labeled selective primers were purchased as a kit through Perkin-Elmer ABI (Applied Biosystems, Foster City, CA). Using this system, AFLP fragments were resolved on a polyacrylamide gel by an ABI 377 gene sequencer. Fluorescent labels were excited by a laser and band emissions were analyzed in the form of an electropherogram where peaks represent individual bands. The sizing of fragments was particularly robust because a DNA ladder was loaded with every sample into the gel. To test for the reproducibility of fingerprints, DNA was extracted from a single isolate on three separate occasions approximately 3 months apart and subjected to the aforementioned protocol.

Data analysis. Isolates with identical multilocus AFLP fingerprints were considered to be members of the same clonal lineage and only a single representative was used for analysis. Because AFLP markers can only be scored confidently for presence (1) or absence (0), allele frequencies were estimated based on the assumption that populations under investigation meet the criterion for Hardy-Weinberg equilibrium, and that loci have only one "present" allele. The term population refers to all samples taken from a single field during a single year.

Genetic diversity within single populations was assessed by calculating the average number of polymorphic bands and estimating the average heterozygosity. Fixation indices were calculated according to methods of Weir and Cockerham (8) for populations from the same site over multiple years and among populations in Michigan using the program tools for population genetic analysis (TFPGA) (M. P. Miller, Northern Arizona University, Flagstaff). Confidence intervals for F statistics at the 95% confidence level were generated by bootstrapping at 1,000 iterations. The program NTSYS-pc version 2.02k (Exeter Software, Setauket, NY) was used to construct a similarity matrix from the presence/absence (1/0) data. Cluster analysis using the unweighted pair group with arithmetic averages (UPGMA) method was performed on the matrix and a tree was generated to give a visual representation of isolate similarity. Excoffier's ARLEQUIN program (L. Excoffier, University of Geneva) was used to assess population differentiation using a phenetic approach termed analysis of molecular variance (AMOVA), which allows for total genetic variation to be partitioned within and among populations using a classical analysis of variance (ANOVA).

RESULTS

Phenotypic results. Five isolates were recovered in 1997 from five different farms (four A1 and one A2 CT). One isolate was fully insensitive to mefenoxam, whereas the other four were fully sensitive. These findings prompted the extensive sampling conducted in 1998 in which 523 isolates (473 from cucurbits and 30 from bell pepper) were collected from 14 farms. A frequency histogram plotting percent growth of control on 100 ppm of mefenoxam-amended media versus number of isolates revealed a trimodal distribution (3). Putative MS categories were assigned based on these groupings with sensitive (S) <30% growth of control, intermediately sensitive (IS) between 30 and 90% growth of control, and insensitive (I) >90% growth of control. In vitro crosses between isolates representative of the different putative sensitivity categories (S × S, I × S, IS × S, and IS × IS) resulted in progeny sets not significantly different than expected for insensitivity inherited as a single incompletely dominant gene unlinked to CT ($P = 0.05$) (3). In 1998, 55% of the isolates were sensitive to mefenoxam, 32% were intermediately sensitive, and 13% were fully insensitive to mefenoxam. A1 and A2 CTs were recovered in a ratio of approximately 1:1 in 8 of the 14 farms. Oospores were detected in naturally diseased cucurbit fruit from four farms, and 223 oospore progeny were recovered and germinated from a single diseased cucumber. All six possible MS × CT combinations were detected in this naturally occurring oospore progeny set (3).

In planta studies using sensitive, intermediately sensitive, and fully insensitive *P. capsici* isolates supported the in vitro screening categories, with sensitive isolates causing no disease on mefenoxam-treated plants, intermediately sensitive isolates being slowed by mefenoxam, and fully insensitive isolates showing no difference in the ability to colonize host tissue between treated and untreated plants at three times the field rate. All the progeny isolates were pathogenic on untreated pumpkin plants (K. H. Lamour and M. K. Hausbeck, *unpublished data*).

Sixty-three mefenoxam insensitive (18% intermediate and 82% fully insensitive) isolates were recovered from a single southwest Michigan field in 1998. Field experiments were conducted in this field during 1999 and 2000, testing alternative cultural control strategies, and no mefenoxam was applied. Two hundred isolates were recovered from this site over the course of the 1999 season and 34 isolates at the beginning of the 2000 season. Of the 200 isolates recovered in 1999 from this field, 141 had unique AFLP genotypes. Seventy percent of these were fully insensitive to mefenoxam, 28% were intermediately sensitive, and 2% were sensitive. In 2000, 15% of the isolates were intermediately sensitive and 85% were fully insensitive. A single fully insensitive clonal lineage rose in frequency over the course of the 1999 season and comprised 20% of the total number of samples recovered (4).

During 1999 and 2000, approximately 2,500 isolates were recovered from farms in Michigan. Both the A1 and A2 CTs were present in every field sampled, and mefenoxam insensitivity was detected in the majority of farms that had a history of mefenoxam use.

Genetic results. Nine populations from the four major vegetable production areas of Michigan were analyzed with the AFLP procedure ($N = 641$). AFLP analysis resolved a total of 94 clearly discernable markers when considering all the isolates together. No single isolate or group of isolates from a single location contained all 94 markers. The total number of AFLP loci in a single population ranged from 68 to 80. Seventeen (18%) fragments were fixed for the present state across all populations, 12 (13%) fragments were polymorphic in all populations, and 65 (69%) were fixed for presence or absence in some populations and polymorphic in others. The number of polymorphic bands within a single population ranged from 37 to 46 with estimated heterozygosities ranging from 0.18 to 0.22. Clonal reproduction was significant within single fields over the course of the growing season. For example, genotypic diversity in a single field ranged from 100% at the beginning of the growing season (seedling stage) to <30% at the time cucurbit fruit were ready for harvest (4). When considering all nine populations, genotypic diversity ranged from 42 to 96% with an average of 74% of the isolates in any sample set having unique genotypes. Although clonal reproduction was significant within single fields within years, no clones were recovered from single fields between years or among fields separated by at least 1 km. Fixation indices (ϕ_{ST}) between the

populations sampled on consecutive years were very close to zero, indicating that gene diversity was not measurably impacted by genetic drift (5). The overall estimated ϕ_{ST} for populations from different locations was 0.35, indicating that approximately 35% of the total genetic diversity present in Michigan *P. capsici* populations is found among populations and 65% is found within any one population. AMOVA partitioned genetic diversity among (40%) and within (60%) populations. The similarity tree based on UPGMA cluster analysis clearly showed that isolates from the same site sampled over years branched from the same node, with no clustering of isolates based on the year of sampling. Cluster analysis also clearly showed that populations separated geographically branched from population-specific nodes (5).

DISCUSSION

During the past 10 years, Michigan has experienced a steady increase in the incidence of root, fruit, and crown rot on cucurbits

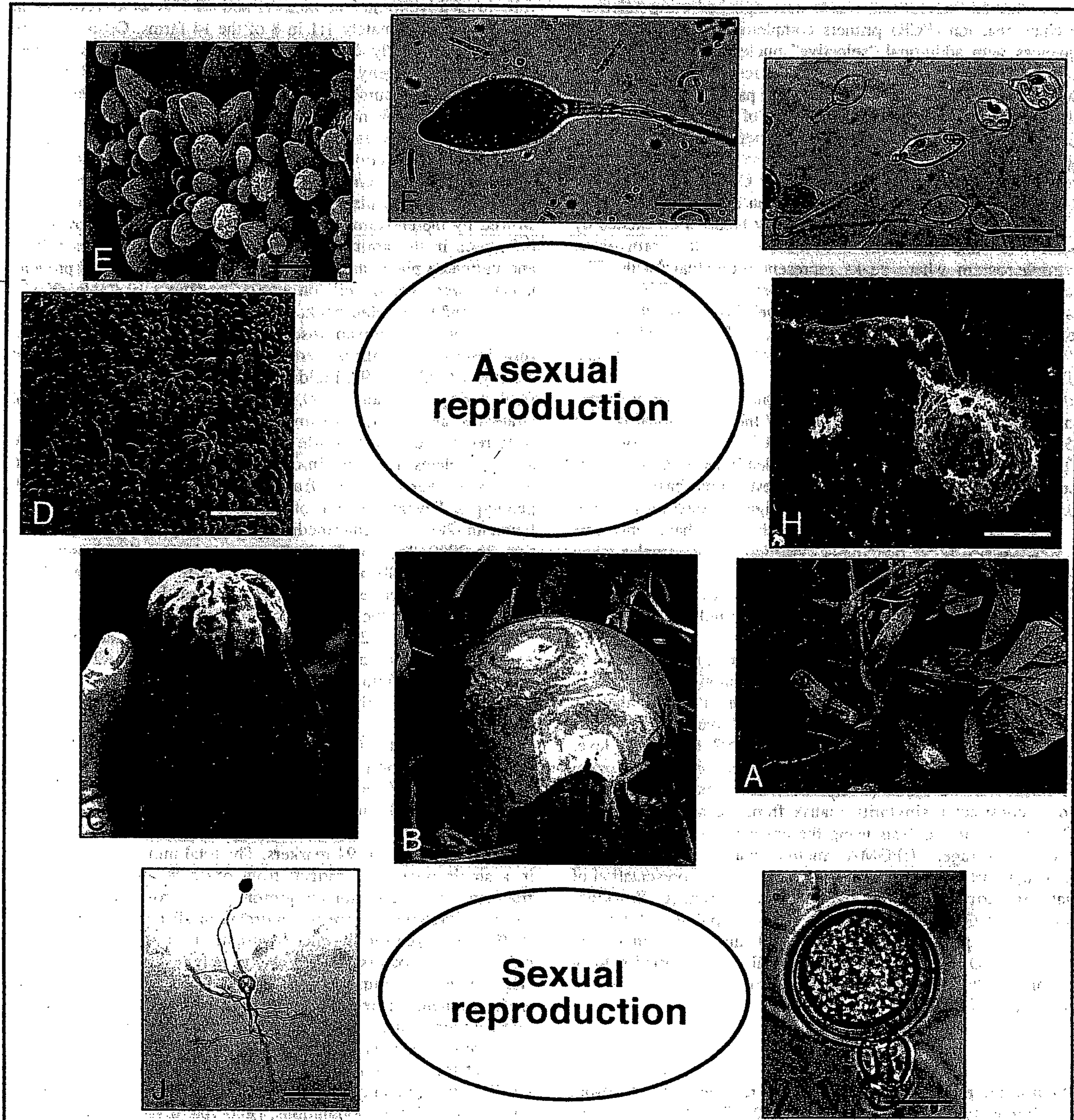

Fig. 1. Spore types and signs of infection caused by *Phytophthora capsici* on cucurbit fruit. **A,** infected cucumber, **B,** pumpkin, and **C,** acorn squash fruit. **D,** Scanning electron microscope (SEM) photo of an infected cucumber showing tufts of sporangia produced on the surface of the fruit (Bar = 300 µm). **E,** Close-up of a single tuft of sporangia (Bar = 30 µm). **F,** Typical papillate sporangium with a long pedicel (Bar = 20 µm). **G,** Zoospores exiting sporangia after immersion in water (Bar = 50 µm). **H,** SEM photo of a single encysted zoospore that germinated and directly penetrated the epidermis of a cucumber fruit (Bar = 4 µm). **I,** Typical amphigynous oospore (Bar = 10 µm). **J,** A germinating oospore with multiple germ tubes and a terminal sporangium (Bar = 100 µm).

caused by *P. capsici*. Rotation to nonsusceptible hosts, in conjunction with cultural and chemical control strategies, have not provided economic control. Correspondence with other vegetable pathologists suggests that this phenomenon is not confined to Michigan, and a similar increase in control failures due to blight by *P. capsici* is being reported throughout the United States.

Investigation of the inheritance of MS demonstrated that MS is inherited as a single incompletely dominant gene unlinked to CT. In 1998, all six possible MS × CT combinations were present in single fields and insensitivity to mefenoxam was common in Michigan. Typical amphigynous oospores were observed in *P. capsici*-infected cucurbit fruit from multiple locations, and oospore progeny from a single naturally infected fruit showed segregation for MS and CT. These findings strongly support the hypothesis that sexual reproduction is occurring in the field, and also suggest that sexual recombination may directly generate progeny fully insensitive to mefenoxam. Tracking a single mefenoxam insensitive population over 2 years in the absence of mefenoxam selection pressure suggests that costs associated with mefenoxam insensitivity are minimal.

Estimates of average heterozygosity and polymorphism indicate surprisingly high levels of gene and genotypic diversity in all the populations of *P. capsici* analyzed. Tracking a single population through an entire growing season showed that asexual reproduction plays a significant role in disease development within a single season. Sampling single fields over consecutive years suggested that clones do not survive Michigan winters and that oospores are the primary survival propagule. Estimation of fixation indices for samples from the same site over consecutive years suggested that there was not a significant reduction in genetic diversity between growing seasons. This implies that populations are large enough to withstand dramatic effects of genetic drift. Cluster analysis revealed unambiguous groups corresponding to geographical locations with regional populations showing more similarity overall than populations from different regions. Population pairwise fixation indices corroborated this finding. The estimated overall fixation index and AMOVA are in agreement with both, suggesting that most (approx 60%) of the total genetic variability in Michigan is found within any one population, but that a relatively large component (40%) of genetic variability is found among populations.

Recommendations based on our findings are as follows: (i) the fungicide mefenoxam may be of limited usefulness because insensitivity appears to be selected for rapidly and is unlikely to decrease when mefenoxam selection pressure is removed; (ii) fields with epidemics are likely to harbor oospores for an extended amount of time (at least 5 years), and this factor must be considered before replanting to susceptible hosts; and (iii) factors that may contribute to the introduction of *P. capsici* into uninfested fields (e.g., drainage ditches between farms, irrigation ponds, and the dumping of culls) need to be considered and if possible avoided, because once an epidemic is established we have found no evidence that the population will become extinct in an agriculturally meaningful time period.

From an evolutionary perspective, it is clear that *P. capsici* has successfully colonized a number of geographical locations in Michigan and that each of the populations sampled thus far have similarly high levels of genetic variability. The genetic stability of single populations over multiple years, the high fixation indices between even geographically close populations (1 km), and the clear structuring based on UPGMA cluster analysis all suggest that long-distance dispersal of inoculum is not common and that geographically isolated populations are also genetically isolated. It appears that the sexual stage of the *P. capsici* life cycle plays a significant role in survival as well as maintaining both genic and genotypic diversity, and has likely played a key role in the evolution of mefenoxam insensitivity. The combination of high levels of genetic variability, thick-walled oospores, and polycyclic asexual disease development make *P. capsici* a formidable pathogen (Fig. 1). This work underscores the need for management strategies aimed at preventing the spread of *P. capsici* to uninfested field sites and suggests that management strategies aimed at limiting spread within a single season may be the only option for growers with *P. capsici*-infested fields.

ACKNOWLEDGMENTS

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Worksheet 3-A(17)(e). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. <u>You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative</u>. Use additional pages as need.

<u>For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed</u>. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: <u>Crop Rotation, Fallow</u> **Study:** <u>Investigating the impact of crop rotation on the genetic structure of *Phytophthora capsici.*</u>

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted __X__
1b. Township caps _____
1c. Alternative not acceptable in consuming country _____
1d. Other (Please describe) _____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete *Section II.*

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Worksheet 3-A(17)(e). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. **Is the study on EPA's website?** Yes ____ No X

   1a. **If not on the EPA website, please attach a copy.**

2. **Author(s) or researcher(s)** K.H. Lamour
   M.K. Hausbeck

3. **Publication and Date of Publication** submitted for publication in Fungal Genetics and Biology, 2002

4. **Location of research study** Michigan, USA

5. **Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.**
   Crop Rotation, Fallow

6. **Was crop yield measured in the study?** Yes ____ No X

7. **Describe the effectiveness of the alternative in controlling pests in the study.**
   Crop rotation was not effective because the oospore is long lived in Michigan soils.

8. **Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?**
   The results of this study are directly applicable since the research was conducted in Michigan, USA.

Investigating the impact of crop rotation on the genetic structure of *Phytophthora capsici*

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ABSTRACT

Lamour, K. H. and Hausbeck, M. K. 200-. Investigating the impact of crop rotation on the genetic structure of *Phytophthora capsici*. Fungal Genetics and Biology _:_-_

Phytophthora capsici isolates (N = 104) recovered from a single field planted to cucumbers (1998), corn (1999 and 2000), and tomatoes (2001) were screened for compatibility type, mefenoxam sensitivity, and AFLP profiles. Eighty-nine percent of the isolates had unique genetic profiles with 60% of the AFLP markers polymorphic. The ratio of A1:A2 compatibility types was $\approx$1:1 and the frequency of mefenoxam insensitive isolates was similar between years. No clonal lineages survived between 1998 and 2001 and the pool of phenotypic and genetic diversity remained essentially intact. This suggests that (i) the site harbors a discrete outcrossing population with little immigration, (ii) two years rotation to corn did not significantly reduce the effective population size, and (iii) cropping to tomatoes did not significantly impact the overall genetic structure of *P. capsici* at this location. The importance of sex in maintaining diversity and allowing survival within a natural population of *P. capsici* is discussed.

Index descriptors: sex, recombination, genetic drift, host selection, migration, AFLP, population genetics, temporal variation.

INTRODUCTION

Phytophthora capsici has been responsible for significant losses to vegetable producers in the United States during the last century (1, 7-9, 13, 19, 21, 22). In Michigan, *P. capsici* causes root, crown, and fruit rot on cucumbers, squash, pumpkins, tomatoes, and peppers and the incidence and severity of disease have increased significantly in the last ten to fifteen years. Control strategies employed by Michigan vegetable producers include planting at well-drained sites, crop rotation to non-susceptible hosts for at least two years, and the application of fungicides. None of the above control strategies have provided economic control under optimal environmental conditions for disease development. The phenylamide fungicide (PAF) mefenoxam, or the similarly acting compound metalaxyl, has been used by some growers. Although PAFs are fungistatic to sensitive isolates of *P. capsici*, a significant problem is that many populations of *P. capsici* in Michigan and elsewhere exposed to metalaxyl or mefenoxam have adapted to PAF selection pressure and the efficacy of PAF's in these populations may be greatly reduced (2, 3, 10, 11, 17, 18).

Phytophthora capsici is an outcrossing diploid organism that requires the presence of both A1 and A2 compatibility types to complete the sexual stage and produce oospores (4). Oospores are thick-walled and appear to play an important role in survival. Asexual reproduction is completed by a coenocytic mycelial thallus able to produce sporangia on the surface of infected tissue. Sporangia are borne on long caducous pedicels and may germinate directly or indirectly. Sporangia germinate indirectly when immersed in free water and produce 20 to 40 motile zoospores. As is the case with many members of the genus *Phytophthora*, excess moisture favors epidemic development (20).

Reports on the spatiotemporal genetic structure of *P. capsici* in Michigan suggest that epidemics are initiated by dormant genetically diverse inoculum and that movement between geographically separated locations is not common (12). Clonal reproduction may be significant within a single growing season, but it appears that the perpetuation of clonal lineages is limited to single fields and single growing seasons (12).

Previous temporal studies were conducted on Michigan *P. capsici* populations infecting cucurbit hosts over the course of a single growing season or among years separated by a single winter (eg; November to March) and may not reflect the dynamics of *P. capsici* over longer periods of time (eg; a typical two year rotation) or among diverse hosts. In the present investigation we test the hypothesis that *P. capsici* can survive a thirty month non-host period via dormant genetically diverse propagules. In addition, we investigated the effects of selection among cucurbit and solanaceous hosts, genetic drift, and migration on population structure.

MATERIALS AND METHODS

Isolate recovery: Tomato plants and cucumber fruit exhibiting typical signs and symptoms of infection by *P. capsici* were collected from a vegetable production field located in south central Michigan that was planted to pickling cucumbers in 1998, corn in 1999 and 2000, and processing tomatoes in 2001. Both the cucumbers and tomatoes were planted on flat ground and the field was irrigated via a pivot irrigation system supplied with water from a well. At the time of harvest in 1998, the majority of the cucumber fruit present in this field had obvious signs and symptoms of disease ranging from discrete water-soaked lesions to being entirely covered with a white, powdery, layer of sporangia. Other than the fruit, the plants appeared to be healthy with no symptoms of disease on foliage, vines, or stems. Close inspection indicated that a limited number of plants (< 5%) scattered throughout the field were stunted.

In 2001, the most frequent above-ground symptom on tomatoes infected with *P. capsici* was stunting with a small number of the infected plants showing wilt symptoms. Foliar lesions were not observed. Plants were recovered prior to fruit being set and the incidence of fruit infection was not determined. The crown area of infected plants was brown-black. Infected plants often had a brown crumbly epidermis from the soil line to the tap root and a significant reduction in feeder roots. In some cases, plants with infected tap roots had numerous adventitious roots above the point of infection. During both 1998 and 2001 diseased plant material was collected in haphazard fashion throughout the field.

Infected cucumbers were snapped in half by hand and a small section (c. 1 cm^2) of tissue removed from beneath the cuticle. The root and crown area of infected tomato plants were rinsed with tap water and patted dry with paper towels before a section of tissue at the edge of an expanding lesion was removed. Tissue was not surface sterilized prior to isolation. Isolations were made onto BARP (benomyl 25 ppm, ampicillin 100ppm, rifampicin 30ppm, and pentachloronitrobenzene 100ppm) amended UCV8 (840 ml distilled water, 163 ml unclarified V8 juice, 3 g $CaCO_3$, and 16 g agar) plates. Plates were incubated at room temperature in the dark for 2 to 3 days before colonies were transferred. Procedures for obtaining single zoospore isolates were as previously described (10). Single zoospore cultures were maintained on RA (rifampicin 30 ppm, ampicillin 100 ppm)-UCV8 plates and transferred bi-monthly. For long-term storage, a 7-mm plug of expanding mycelium from each culture was placed into a 1.5 ml microfuge tube with one sterilized hemp seed and 1 ml of sterile distilled water (SDW). Isolates were then incubated for 2 to 3 weeks at 23 to 25°C before being stored at 15°C.

Compatibility type and mefenoxam sensitivity determination: Agar plugs from the edge of an expanding single-zoospore colony were placed at the center of UCV8 plates approximately 2 cm from field isolates OP97 (A1) and SP98 (A2) and incubated in the dark at 23 to 25°C for 3 to 6 days. Following incubation, compatibility type was determined.

Agar plugs from the edge of actively expanding single-zoospore colonies were placed at the center of 100 x 15 mm UCV8 plates amended with 0 or 100 ppm mefenoxam (Ridomil Gold EC, Novartis, Greensboro, NC; 48% AI, suspended in SDW; added to UCV8 cooled to 49°C). Inoculated plates were incubated at 23 to 25°C for 3 days and colony diameters measured. Percentage growth of an isolate on amended media was calculated by subtracting the inoculation plug diameter (7-mm) from the diameter of each colony and dividing the average diameter of the colony on amended plates by the average diameter of the colony on unamended control plates. All tests were conducted at least twice. An isolate was scored as sensitive (S) if growth at 100 ppm was < 30% of the control, intermediately sensitive (IS) if growth was between 30 and 90% of the control, and insensitive (I) if growth was > 90% of the control (10).

DNA extraction and AFLP fingerprinting: Bacterial contamination was avoided by using a modified Van Teigham cell (4). The uppermost portion of a 7-mm plug of mycelium was placed onto the surface of RA-WA plates (rifampicin 30 ppm, ampicillin 100 ppm, 1000 ml distilled water, and 16 g agar) and an autoclaved cap from a 1.5 ml microfuge tube was placed over the plug which forced the isolate to grow through the amended medium. Isolates were incubated in the dark for 2 to 3 days before two 7-mm plugs were transferred to approximately 15 ml of RA-UCV8 broth in 100 x 15 mm Petri dishes and incubated in the dark for three days at 23 to

25°C. Mycelial mats were washed with distilled water and dried briefly under vacuum before being frozen to -20°C and lyophilized.

Lyophilized mats were ground with a sterile mortar and pestle. Whole genomic DNA from approximately 50 mg of ground mycelium was extracted using a QIAGEN Dneasy Plant Mini Kit (QIAGEN Inc., Valencia, CA) according to the manufacturers directions. DNA was quantified using Nucleic Acid QuickSticks (CLONTECH, Palo Alto, CA) according to the manufacturer's directions or on 1.5% agarose gels. Approximately 100 ng of DNA was then subjected to a restriction/ligation reaction, pre-selective amplification, and selective amplifications using the PCR core mix, adaptor sequences, core primer sequences and fluorescence labeled primers provided in the AFLP™ Microbial Fingerprinting Kit (Perkin-Elmer Corp., Foster City, CA) and performed exactly as described in the PE/ABI AFLP Microbial Fingerprinting protocol part # 402977 Rev A (23). All PCR reactions were performed using an MJ Research Minicycler (MJ Research Inc., Waltham, MA) in 0.2 ml tubes according to the cycling parameters outlined in the microbial fingerprinting protocol.

An initial optimization set of reactions was performed using pre-selective products from *P. capsici* isolate OP97 which was isolated from a cucumber fruit in 1997 (10). Amplifications with the selective primers EcoRI-AA, AC, AG and AT were performed in all 16 combinations with the MseI-CA, CC, CG and CT selective primers. EcoRI selective primers were labeled at the 5' end with either carboxyfluorescein (FAM), carboxytetramethyrhodamine (TAMRA), or carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein (JOE) fluorescent dyes. The fluorescent dyes were excited by laser radiation and visualized by their characteristic absorption-emission frequencies. Only the fragments containing an EcoRI restriction site were resolved.

Selective amplification AFLP products and a carboxy-X-rhodamine (ROX) size standard were loaded into each lane on a denaturing polyacrylamide gel and the fragments resolved in an ABI Prism 377 DNA Sequencer. Results were prepared for analysis in the form of electropherograms using GeneScan Analysis software (PE/ABI). AFLP fragments were scored manually as present = 1 or absent = 0 using Genotyper (PE/ABI). Only DNA bands which consistently exhibited unambiguous presence/absence profiles were scored.

In order to assess the reproducibility of AFLP profiles, a single isolate, OP97, was subjected to the aforementioned protocol using three optimal primer pair combinations on three separate occasions approximately three months apart.

Clone detection: AFLP fragments for each field isolate were scored for presence or absence, and the binary data matrix was converted to a similarity matrix with the program NTSYSpc version 2.02k (Exeter Software, Setauket, NY). Unweighted pair group method with arithmetic averages (UPGMA) cluster analysis was performed on the similarity matrix and a tree was generated. Isolates showing complete homology at all loci were considered to be members of the same clonal lineage, and except for a single representative isolate (referred to as a clone) were excluded from population genetic analysis (15).

Population genetic analysis: Sample sets collected from single fields during a single year were considered a population. Populations were assumed to be in Hardy-Weinberg equilibrium, and each AFLP locus was assumed to be di-allelic and selectively neutral. The program 'Tools for population genetic analysis' (TFPGA) (Miller, M. P., Northern Arizona Univ., Flagstaff, AZ) was used to assess genetic diversity within each population on the basis of estimated average heterozygosity (16) and the proportion of polymorphic loci at the 95% level (6), and to calculate pairwise and overall fixation indices (F-statistics) according to the methods of Weir and Cockerham (24). Confidence intervals for F-statistics at the 95% confidence level were generated by boot-strapping using 1000 iterations. Estimates of the percent polymorphic loci and estimated average heterozygosity were calculated based on the 68 AFLP markers resolved.

The fixation index, as described by Wright, equals the reduction in heterozygosity expected with random mating at any one level of a population hierarchy relative to another, more inclusive level of the hierarchy (25). Weir and Cockerham's approach to estimating fixation indices attempts to correct for the effects of sampling a limited number of organisms from a limited number of populations and is reported as Φ_{ST} instead of F_{ST} (24). Theoretically, the fixation index has a minimum of 0 (no loss of heterozygosity between the populations compared) and maximum of 1 (indicating fixation for alternative alleles in different populations or a total loss of heterozygosity), but, as discussed by Hartl and Clark (6), the observed maximum is usually much less than 1. Wright has suggested the following qualitative guidelines for the interpretation of fixation indices: the range 0 to 0.05 indicates little genetic differentiation, 0.05

to 0.15 indicates moderate genetic differentiation, 0.15 to 0.25 indicates great genetic differentiation, and values above 0.25 indicate very great genetic differentiation.

Using the program NTSYS-pc, the combined 0/1 data matrix for isolates from all populations was used to construct a genetic similarity matrix of all possible pairwise comparisons of individuals within and among populations using Jaccard's similarity coefficient: $GS(ij) = a/(a + b + c)$. $GS(ij)$ is the measure of genetic similarity between individuals i and j, where a is the number of polymorphic bands shared by i and j, b is the number of bands present in i and absent in j, and c is the number of bands present in j but absent in i. A tree was constructed using UPGMA cluster analysis to provide a graphic representation of the relationships among isolates.

Genetic structure was also examined by analysis of molecular variance (AMOVA) using the ARLEQUIN software package (Excoffier, L., University of Geneva, Geneva). The AMOVA analysis was used to partition the variance in banding patterns within and among the populations. Significance values were assigned to variance components on the basis of a set of null distributions generated by a permutation process which randomly assigned individuals to populations and drew 1000 independent samples.

RESULTS

Isolate recovery: In 1998, 141 isolates of *P. capsici* were recovered from infected cucumber fruit. Phenotypic characterization of all 141 isolates and genetic characterization of 57 isolates has been reported previously (10, 12). Here we report only on the 57 isolates characterized genetically. In 2001, 47 isolates of *P. capsici* were recovered from infected tomato plants.

Genetic diversity, compatibility type, and sensitivity to mefenoxam: Evaluation of the 16 EcoRI + 2/MseI + 2 selective primer pair combinations indicated that EcoRI + AC/ MseI + CA gave the most clearly resolved fragment profile and was used to amplify genomic DNA from all isolates in both the 1998 and 2001 sample sets. This primer combination resulted in 68 clearly resolved fragments of which 42 fragments were polymorphic in 1998 and 45 were polymorphic in 2001 (Table 1). All 68 fragments were present in both 1998 and 2001 and no novel fragments were detected among years. AFLP profiles for isolate OP97, generated from separate DNA extractions on three separate occasions over a one year period, resulted in identical banding patterns with the only difference being minor changes in the intensity of the electropherogram signal. Occasionally individual reactions resulted in poorly resolved fingerprint profiles (eg, low intensity of signal) and were repeated until signals were deemed optimal.

In 1998, there were five clonal lineages detected with three lineages comprised of two members and two lineages with three isolates each. In 2001, there were four clonal lineages detected with two members each. No members of the same clonal lineage were detected among the isolates collected in 1998 and 2001. The ratio of A1:A2 isolates was 29:21 in 1998 and 21:22 in 2001 (Table 2). These numbers approximate the 1:1 ratio expected for a randomly outcrossing diploid organism. The percentage of isolates falling into the three mefenoxam sensitivity categories was 40% and 44% sensitive, 56% and 38% intermediately sensitive, and 4% and 18% fully insensitive for 1998 and 2001 respectively (Table 2).

Temporal dynamics: The fixation index (Φ_{ST}) among the populations of *P. capsici* recovered in 1998 and 2001 was 0.05 with a standard deviation of 0.01. This indicates that very little genetic differentiation, or loss of heterozygosity, occurred between years at this location. The number and identity of bands polymorphic at the 95% level and the estimated average heterozygosity (0.17 for both years) remained relatively similar over time (Table 1). AMOVA analysis partitioned 3% of the total variability among years indicating that 97% of the variation found in 1998 was also found in 2001 (Table 3). These data suggest that there was enough out-crossing and survival of the resulting recombinant progeny at this location to maintain genic diversity. UPGMA analysis showed that unique genotypes were between 76 and 96% similar and that isolates from 1998 and 2001 were dispersed randomly throughout the tree (Fig 1). There was no grouping of isolates based on year or host.

Discussion

In this study we investigated the genetic structure *P. capsici* infecting cucumbers and tomatoes separated by two years of crop rotation to corn. Previous studies indicate that genetic diversity is high in natural populations of *P. capsici* in Michigan and that the pool of AFLP markers resolved from the isolates at this location is unique (12). By tracking the changes in the identity and frequency of the AFLP markers over time we were able to gain novel insight into the survivability of *P. capsici* at a naturally infested site and to begin deciphering the impact of crop rotation on *P. capsici*'s population structure.

There are three major evolutionary forces that could have changed the genetic structure of *P. capsici* at this location between 1998 and 2001; genetic drift, selection, and migration (5, 14). A significant pressure by any one of these should be discernable in the patterns of genetic diversity recovered among years. Genetic drift refers to the random sampling process that occurs within small populations over time (6). If only a small number of *P. capsici* propagules survived between the epidemics in 1998 and 2001, then there should be significant changes in the respective frequencies of neutral genetic markers just due to chance. In particular, it's expected that the total genetic diversity recovered in 2001 would be a subset of that recovered in 1998 because some rare markers would be missed in the sampling (= survival) process. An in-depth summary of genetic drift is not possible in this context, but it is clear that there was not a significant reduction in the total genetic diversity among years at this location. This is illustrated by the fixation index estimate of 0.05 and the AMOVA analysis which indicate that between 95 and 97% of the genetic diversity found in one year was also found in the other. This suggests that enough propagules survived between 1998 and 2001 to withstand the differentiating effects of genetic drift.

There are many environmental forces able to exert selective pressures on living organisms. In the case presented here, the different susceptible hosts planted at this location have the potential to select for different genetic characteristics in the *P. capsici* isolates attempting to cause infection. If only a subset of the isolates able to infect cucumbers were able to successfully infect tomatoes then an incomplete sample of the total genetic diversity would be represented by the infecting propagules. Here again we would expect a subset of the genetic diversity recovered in 1998 to be recovered in 2001. In this case, differentiation is not due to random sampling of a small population, but to the non-random nature of the sampling process (eg; *P. capsici* isolates possessing specific genetic characters or constellations of characters are more successful) that occurs under selection. Not only was there no appreciable decrease in the total amount of genetic diversity between 1998 and 2001, but there is no indication that *P. capsici* isolates infecting tomatoes are more similar to each other than they are to isolates recovered from cucumbers. This is illustrated by the genetic similarity tree which showed no increased genetic similarity (clustering) based on host or year.

We were also interested in the contribution of immigrants to the epidemic in 2001. A previous investigation of the genetic structure of *P. capsici* populations at diverse locations in Michigan suggested that movement among locations was infrequent. Isolates from separate geographical locations were unambiguously more similar to each other, even when comparing fields separated by 1 km (12). If there were significant movement of *P. capsici* propagules into this field, then it is expected that the frequencies of the AFLP markers would differ among years and that novel markers would be introduced in 2001. Marker frequencies did not fluctuate appreciably between years and there were no new AFLP markers detected in 2001. In addition, the frequency of mefenoxam insensitivity remained relatively stable. This suggests that immigration did not contribute significantly to the epidemic occurring in 2001.

In conclusion, it appears that these data support the hypothesis that *P. capsici* survives non-host periods as genetically diverse oospores. Furthermore, this population appears to have maintained its genetic diversity through a 30 month non-host period. How long viable propagules remain in a field following an epidemic is still open to speculation, but it is clear that a typical two year rotation may not ensure against another epidemic. Since it appears that *P. capsici* may remain for extended periods after being introduced and that migration is not a frequent event, then it may be helpful to decipher the mechanisms by which it spreads and develop strategies to limit introduction into new sites.

ACKNOWLEDGMENTS

This work was funded by the Michigan Agricultural Experiment Station, Michigan State University Extension, Michigan Department of Agriculture, Michigan Farm Bureau (GREEEN cooperative), Pickle and Pepper Research Committee, Pickle Packers International, Inc. and the Pickle Seed Research Fund, Pickle Packers International. We thank M. Bour, C. Hunter, J. Jabara, P. Tumbalam, E.Webster, and J. Woodworth for competent laboratory assistance.

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Table 1. Estimates of genetic diversity within populations of *Phytophthora capsici* recovered from cucumbers (1998) and tomatoes (2001) planted at the same location in south central Michigan

Year	No. of isolates	Unique isolates[a]	No. of AFLP bands	No. and percent polymorphic bands	Estimated average heterozygosity
1998	57	50	68	42 (62)	0.17
2001	47	43	68	45 (66)	0.17

[a] Total number of isolates with unique multilocus AFLP profiles.

Table 2: Phenotypic diversity of *Phytophthora capsici* isolates recovered from cucumbers (1998) and tomatoes (2001) planted at the same location in south central Michigan

Year	No. of isolates[a]	Compatibility type/mefenoxam sensitivity[b,c]					
		A1/S	A1/IS	A1/I	A2/S	A2/IS	A2/I
1998	50	10 (.20)	17 (.34)	2 (.04)	10 (.20)	11 (.22)	-
2001	43	7 (.16)	10 (.24)	4 (.09)	12 (.28)	6 (.14)	4 (.09)
Total	93	17 (.18)	27 (.30)	6 (.06)	22 (.24)	17 (.18)	4 (.04)

[a] Total number of isolates with unique multilocus AFLP profiles.

[b] Mefenoxam sensitivity determined by in vitro screening on 100 ppm AI amended media with S = < 30% growth of control (GC), IS = between 30 and 90% GC and I = > 90% GC.

[c] Observed numbers are followed by proportion of total sample size in parenthesis.

Table 3: Results of nested analysis of molecular variance (AMOVA) for *Phytophthora capsici* isolates based on 68 AFLP markers. Variance is partitioned between isolates recovered in 1998 (N = 50) from cucumbers and 2001 (N = 43) from tomatoes at the same location in south central Michigan.

Source of variation[a]	Degrees of freedom	Sum of squares	Variance component	Percentage of variation	P [a]
1998 and 2001					
Among populations	1	17.676	0.232	3.40	<0.0001
Within populations	67	618.386	6.578	96.60	

[a] P = the probability of obtaining a more extreme component estimate by chance alone based on 1000 sampling realizations.

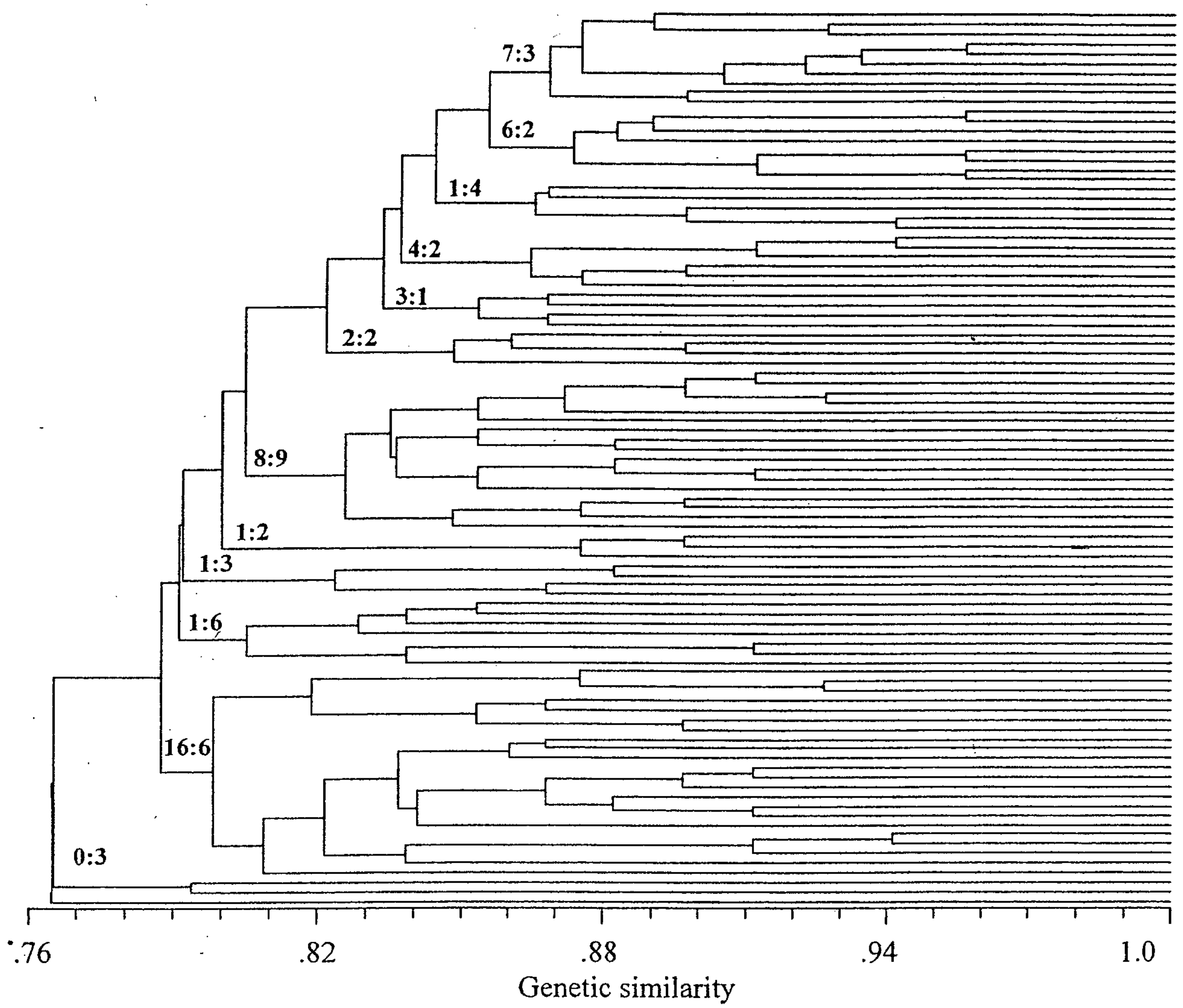

Fig. 1: UPGMA cluster analysis of *Phytophthora capsici* isolates recovered from cucumbers (1998, N = 50) and tomatoes (2001, N = 43) at the same location in south central Michigan based on the Jaccard similarity coefficient using 68 amplified fragment length polymorphism (AFLP) markers. The ratio of isolates recovered in 1998 (cucumber) to the number recovered in 2001 (tomato) within sub-clusters is indicated at major branch points.

Worksheet 3-A(18). Alternatives - Technical Feasibility of Alternatives to Methyl

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative. Use additional pages as need.

For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Endophytes **Study:** UNEP 1995, A-73

Section I. Initial Screening on Technical Feasibility of Alternatives

1. **Are there any location-specific restrictions that inhibit the use of this alternative on your site?**

   1a. Full use permitted ___X___
   1b. Township caps ______
   1c. Alternative not acceptable in consuming country ______
   1d. Other (Please describe) ______

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

Worksheet 3-A(18). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes X No ____

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ____

3. Publication and Date of Publication ____

4. Location of research study ____

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

   Endophytes

6. Was crop yield measured in the study? Yes ____ No ____

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?

   The use of non-pathogenic endophytes to control *Phytophthora capsici* is not proven and cannot be considered a viable alternative at this time.

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Worksheet 3-A(19). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous not effective for your conditions. This worksheet contains 9 questions. <u>You must complete one copy of work each research study you use to evaluate a single methyl bromide alternative</u>. Use additional pages as need.

<u>For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed</u>. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: <u>Flooding, Water Management</u> **Study:** <u>UNEP 1995, UNEP 1998, UNEP 2001, A-74, A-77, B-42, E-74</u>

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted ___X___
1b. Township caps ______
1c. Alternative not acceptable in consuming country ______
1d. Other (Please describe) ______

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If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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Worksheet 3-A(19). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes X No ____

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ______________

3. Publication and Date of Publication ______________

4. Location of research study ______________

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

   Flooding, Water Management

6. Was crop yield measured in the study? Yes ____ No ____

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?

   One of the pathogens mentioned in the study is *Phytophthora* spp., which is a primary problem in Michigan. However, our results differ because our production is in the field rather than the greenhouse. Michigan growers typically use trickle irrigation and raised beds to manage water in order to reduce *Phytophthora* infection. However, these practices are not adequate in Michigan's climate where heavy rains are common. These heavy rainshowers are very conducive to *Phytophthora*, and cannot be controlled.

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Worksheet 3-A(19)(b). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. <u>You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative</u>. Use additional pages as need.

<u>For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed</u>. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: <u>Water Management</u> **Study: <u>The spatiotemporal genetic structure of *Phytophthora capsici* in Michigan and implications for disease management.</u>**

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted ___X___
1b. Township caps ______
1c. Alternative not acceptable in consuming country ______
1d. Other (Please describe) ______

____

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If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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ID# ____________

Worksheet 3-A(19)(b). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. **Is the study on EPA's website?** **Yes** ______ **No** X

   1a. If not on the EPA website, please attach a copy.

2. **Author(s) or researcher(s)** K.H. Lamour
   M.K. Hausbeck

3. **Publication and Date of Publication** Phytopathology 92:681-684, 2002

4. **Location of research study** Michigan, USA

5. **Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.**
   Water Management

6. **Was crop yield measured in the study?** **Yes** ______ **No** X

7. **Describe the effectiveness of the alternative in controlling pests in the study.**
   Despite the use of well-drained fields, conservative irrigation, and planting on raised beds, *Phytophthora capsici* is not adequately controlled.

8. **Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?**
   The results of this study are directly applicable since the research was conducted in Michigan, USA.

The Spatiotemporal Genetic Structure of *Phytophthora capsici* in Michigan and Implications for Disease Management

K. H. Lamour and M. K. Hausbeck

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Accepted for publication 12 February 2002.

Root, crown, and fruit rot caused by *Phytophthora capsici* Leonian is a limiting factor for the production of peppers, tomatoes, and cucurbit crops in Michigan and the United States. Like many species in the genus *Phytophthora*, *P. capsici* has the potential for rapid polycyclic disease development from a limited amount of initial inoculum (6). *P. capsici* produces caducous sporangia that can be spread by wind-blown rain or release 20 to 40 motile zoospores in the presence of free water. The polycyclic phase of disease development is thought to be driven primarily by asexual spore dispersal at a local scale (within and down rows). Sexual reproduction requires both the A1 and A2 compatibility types (CTs) and results in the production of thick-walled oospores. Oospores are thought to serve as the primary survival structure outside of host tissue.

Recommended disease management strategies stress the importance of avoiding excess water in the plant rhizosphere by using well-drained fields, conservative irrigation, and planting on raised beds. Additional recommendations include rotation to nonsusceptible hosts for at least 2 years and the use of fungicides. The phenylamide fungicide (PAF) mefenoxam is a systemic compound with high activity against *P. capsici* and has been used by growers throughout the United States to control *P. capsici*. Insensitivity to PAF has been reported for a number of other oomycetous organisms (*Bremia lactucae*, *P. infestans*, and *P. sojae*, etc.) and appears to be conferred by a single incompletely dominant gene of major effect (1). Growers in Michigan practicing 2+-year rotation in well-drained fields using an array of fungicidal management tools have experienced significant losses to *P. capsici*. Michigan is the number one producer of cucumbers for pickling in the United States and it was at the request of grower groups associated with this industry that research into the epidemiology and reproductive biology of *P. capsici* on cucurbit hosts was initiated.

Although many researchers cite oospores as the most likely propagule for survival outside of host tissue, there have been very few investigations specifically aimed at determining the impact of sexual reproduction in natural populations of *P. capsici*. Our hypothesis was that the sexual stage may play an important role not only in survival but also in the adaptation of *P. capsici* populations to environmental stresses (e.g., fungicides). Our goal was to perform a comprehensive investigation of the phenotypic and genetic diversity present in *P. capsici* populations from the major vegetable production regions of Michigan, with the implicit intention of addressing questions concerning epidemiology, reproductive biology, and the durability of currently recommended management strategies.

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METHODOLOGY

Isolate collection and maintenance. Sampling of diseased fields began at the end of the 1997 growing season and continued through September 2000. In all cases, fields were sampled on a grid with quadrants varying from 40 m^2 to 12 km^2. A limited number of isolates were collected in 1997. In 1998, the strategy was to collect as many samples from as many fields as possible. This strategy was modified in 1999 and 2000 to focus on specific fields. Isolations from diseased plants were made onto selective media and single zoospore cultures were generated according to standard single sporing techniques (3). Isolates were placed into long-term storage (15°C) using a hemp seed/sterile water technique.

Phenotypic characterization. Single zoospore isolates were screened for CT using known A1 and A2 isolates. In vitro screening techniques published for other *Phytophthora* species for assessing sensitivity to mefenoxam were compared and a novel, simple, high dose screen using 100 ppm of mefenoxam-amended V8 agar was found to separate field isolates into three modal distributions that appeared consistent with the expectations of a single incompletely dominant gene governing mefenoxam insensitivity (e.g., sensitive, intermediately sensitive, and fully insensitive). These putative mefenoxam sensitivity (MS) groupings were tested by performing a series of crosses and testing whether the observed progeny sets met the expectations for Mendelian inheritance of a single incompletely dominant gene controlling insensitivity to mefenoxam. Sexual crosses were conducted on unclarified V8 agar plates and incubated for 3 months in the dark. Individual germinated oospores were recovered after 3 months using previously published techniques (2).

The efficacy of this in vitro mefenoxam screening technique was further tested in pumpkin seedlings using progeny from a cross between parents intermediately sensitive to mefenoxam. Nine isolates from each of the three MS categories were screened for pathogenicity on untreated seedlings. Single sensitive, intermediately sensitive, and fully insensitive isolates were then placed onto the unwounded surface of plants treated with either a field rate of mefenoxam, three times the field rate, or distilled water. Lesion diameters on seedling stems were measured after 4 days.

Genetic characterization. Single zoospore isolates were grown in antibiotic-amended V8 broth for 3 days at room temperature. Mycelial mats were washed, frozen, lyophilized, and ground with a sterile mortar and pestle. DNA was extracted with either a Qiagen Dneasy extraction kit (Qiagen, Valencia, CA) or via a cetyltrimethylammonium bromide (CTAB) procedure. A variety

of methods for generating molecular markers were tested for efficacy including isozyme, random amplified polymorphic DNA, and amplified fragment length polymorphism (AFLP). The AFLP technique resulted in a large number of reproducible markers and was chosen to characterize samples of *P. capsici* from Michigan. The AFLP technique involves cutting genomic DNA with moderately rare cutting (*Eco*RI) and frequent cutting (*Mse*I) restriction enzymes, while concomitantly ligating synthetic adaptor fragments of DNA to the sticky ends created by the restriction enzymes (7). The result is a large number of DNA fragments that have ends with known DNA sequences. Amplification of fragment subsets (termed fingerprints) can be accomplished using polymerase chain reaction (PCR) primers complementary to the adaptor sequences with additional "selective" nucleotides. Changing the amount and type of selective nucleotides results in different subsets or fingerprints. Stringent PCR cycling parameters (touchdown technique) are used to ensure the fidelity of the reaction. For the analysis summarized here, adaptor sequences and fluorescent labeled selective primers were purchased as a kit through Perkin-Elmer ABI (Applied Biosystems, Foster City, CA). Using this system, AFLP fragments were resolved on a polyacrylamide gel by an ABI 377 gene sequencer. Fluorescent labels were excited by a laser and band emissions were analyzed in the form of an electropherogram where peaks represent individual bands. The sizing of fragments was particularly robust because a DNA ladder was loaded with every sample into the gel. To test for the reproducibility of fingerprints, DNA was extracted from a single isolate on three separate occasions approximately 3 months apart and subjected to the aforementioned protocol.

Data analysis. Isolates with identical multilocus AFLP fingerprints were considered to be members of the same clonal lineage and only a single representative was used for analysis. Because AFLP markers can only be scored confidently for presence (1) or absence (0), allele frequencies were estimated based on the assumption that populations under investigation meet the criterion for Hardy-Weinberg equilibrium, and that loci have only one "present" allele. The term population refers to all samples taken from a single field during a single year.

Genetic diversity within single populations was assessed by calculating the average number of polymorphic bands and estimating the average heterozygosity. Fixation indices were calculated according to methods of Weir and Cockerham (8) for populations from the same site over multiple years and among populations in Michigan using the program tools for population genetic analysis (TFPGA) (M. P. Miller, Northern Arizona University, Flagstaff). Confidence intervals for F statistics at the 95% confidence level were generated by bootstrapping at 1,000 iterations. The program NTSYS-pc version 2.02k (Exeter Software, Setauket, NY) was used to construct a similarity matrix from the presence/absence (1/0) data. Cluster analysis using the unweighted pair group with arithmetic averages (UPGMA) method was performed on the matrix and a tree was generated to give a visual representation of isolate similarity. Excoffier's ARLEQUIN program (L. Excoffier, University of Geneva) was used to assess population differentiation using a phenetic approach termed analysis of molecular variance (AMOVA), which allows for total genetic variation to be partitioned within and among populations using a classical analysis of variance (ANOVA).

RESULTS

Phenotypic results. Five isolates were recovered in 1997 from five different farms (four A1 and one A2 CT). One isolate was fully insensitive to mefenoxam, whereas the other four were fully sensitive. These findings prompted the extensive sampling conducted in 1998 in which 523 isolates (473 from cucurbits and 30 from bell pepper) were collected from 14 farms. A frequency histogram plotting percent growth of control on 100 ppm of mefenoxam-amended media versus number of isolates revealed a trimodal distribution (3). Putative MS categories were assigned based on these groupings with sensitive (S) <30% growth of control, intermediately sensitive (IS) between 30 and 90% growth of control, and insensitive (I) >90% growth of control. In vitro crosses between isolates representative of the different putative sensitivity categories (S × S, I × S, IS × S, and IS × IS) resulted in progeny sets not significantly different than expected for insensitivity inherited as a single incompletely dominant gene unlinked to CT (P = 0.05) (3). In 1998, 55% of the isolates were sensitive to mefenoxam, 32% were intermediately sensitive, and 13% were fully insensitive to mefenoxam. A1 and A2 CTs were recovered in a ratio of approximately 1:1 in 8 of the 14 farms. Oospores were detected in naturally diseased cucurbit fruit from four farms, and 223 oospore progeny were recovered and germinated from a single diseased cucumber. All six possible MS × CT combinations were detected in this naturally occurring oospore progeny set (3).

In planta studies using sensitive, intermediately sensitive, and fully insensitive *P. capsici* isolates supported the in vitro screening categories, with sensitive isolates causing no disease on mefenoxam-treated plants, intermediately sensitive isolates being slowed by mefenoxam, and fully insensitive isolates showing no difference in the ability to colonize host tissue between treated and untreated plants at three times the field rate. All the progeny isolates were pathogenic on untreated pumpkin plants (K. H. Lamour and M. K. Hausbeck, *unpublished data*).

Sixty-three mefenoxam insensitive (18% intermediate and 82% fully insensitive) isolates were recovered from a single southwest Michigan field in 1998. Field experiments were conducted in this field during 1999 and 2000, testing alternative cultural control strategies, and no mefenoxam was applied. Two hundred isolates were recovered from this site over the course of the 1999 season and 34 isolates at the beginning of the 2000 season. Of the 200 isolates recovered in 1999 from this field, 141 had unique AFLP genotypes. Seventy percent of these were fully insensitive to mefenoxam, 28% were intermediately sensitive, and 2% were sensitive. In 2000, 15% of the isolates were intermediately sensitive and 85% were fully insensitive. A single fully insensitive clonal lineage rose in frequency over the course of the 1999 season and comprised 20% of the total number of samples recovered (4).

During 1999 and 2000, approximately 2,500 isolates were recovered from farms in Michigan. Both the A1 and A2 CTs were present in every field sampled, and mefenoxam insensitivity was detected in the majority of farms that had a history of mefenoxam use.

Genetic results. Nine populations from the four major vegetable production areas of Michigan were analyzed with the AFLP procedure (N = 641). AFLP analysis resolved a total of 94 clearly discernable markers when considering all the isolates together. No single isolate or group of isolates from a single location contained all 94 markers. The total number of AFLP loci in a single population ranged from 68 to 80. Seventeen (18%) fragments were fixed for the present state across all populations, 12 (13%) fragments were polymorphic in all populations, and 65 (69%) were fixed for presence or absence in some populations and polymorphic in others. The number of polymorphic bands within a single population ranged from 37 to 46 with estimated heterozygosities ranging from 0.18 to 0.22. Clonal reproduction was significant within single fields over the course of the growing season. For example, genotypic diversity in a single field ranged from 100% at the beginning of the growing season (seedling stage) to <30% at the time cucurbit fruit were ready for harvest (4). When considering all nine populations, genotypic diversity ranged from 42 to 96% with an average of 74% of the isolates in any sample set having unique genotypes. Although clonal reproduction was significant within single fields within years, no clones were recovered from single fields between years or among fields separated by at least 1 km. Fixation indices (ϕ_{ST}) between the

populations sampled on consecutive years were very close to zero, indicating that gene diversity was not measurably impacted by genetic drift (5). The overall estimated ϕ_{ST} for populations from different locations was 0.35, indicating that approximately 35% of the total genetic diversity present in Michigan *P. capsici* populations is found among populations and 65% is found within any one population. AMOVA partitioned genetic diversity among (40%) and within (60%) populations. The similarity tree based on UPGMA cluster analysis clearly showed that isolates from the same site sampled over years branched from the same node, with no clustering of isolates based on the year of sampling. Cluster analysis also clearly showed that populations separated geographically branched from population-specific nodes (5).

DISCUSSION

During the past 10 years, Michigan has experienced a steady increase in the incidence of root, fruit, and crown rot on cucurbits

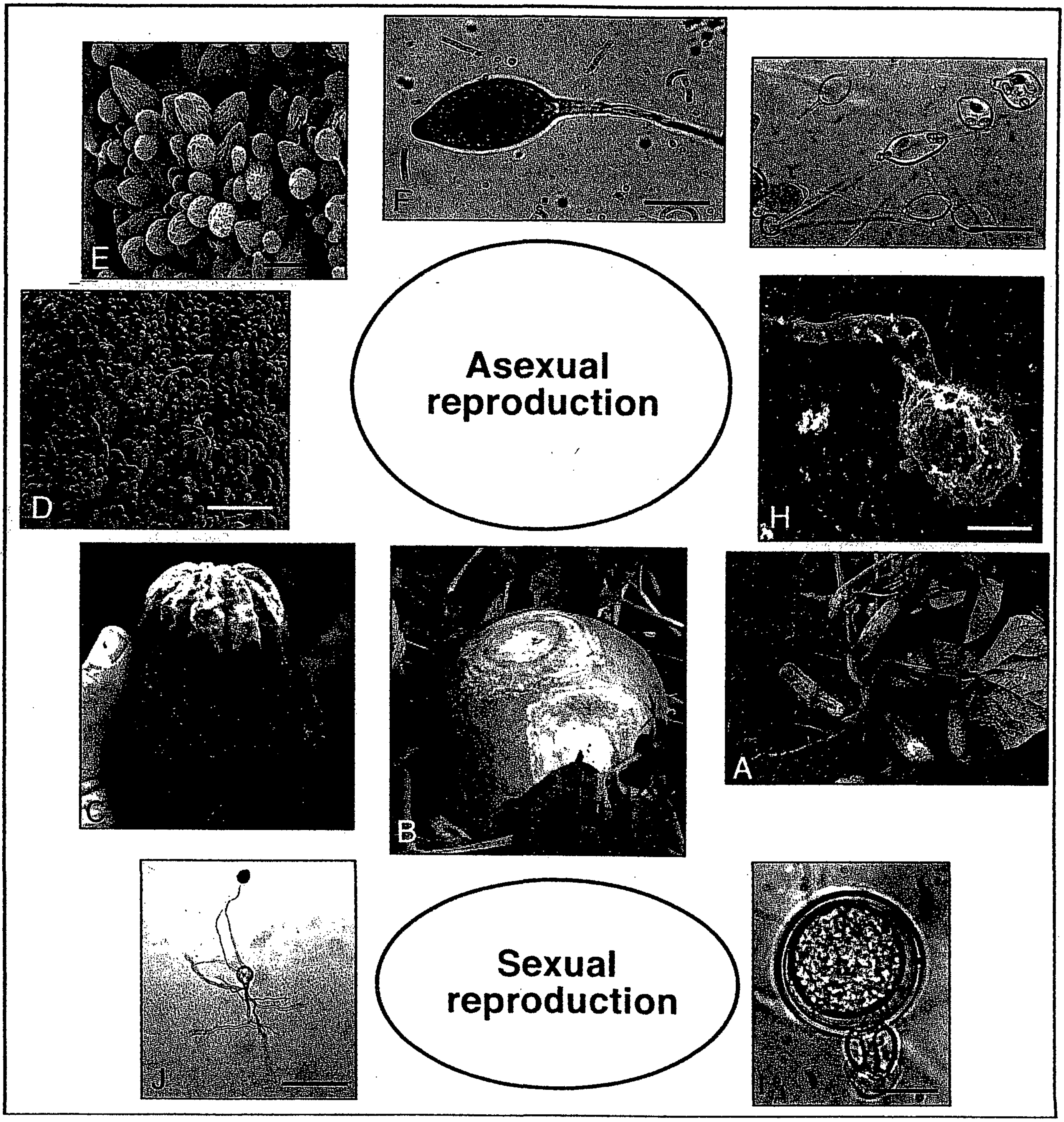

Fig. 1. Spore types and signs of infection caused by *Phytophthora capsici* on cucurbit fruit: **A,** infected cucumber, **B,** pumpkin, and **C,** acorn squash fruit. **D,** Scanning electron microscope (SEM) photo of an infected cucumber showing tufts of sporangia produced on the surface of the fruit (Bar = 300 μm). **E,** Close-up of a single tuft of sporangia (Bar = 30 μm). **F,** Typical papillate sporangium with a long pedicel (Bar = 20 μm). **G,** Zoospores exiting sporangia after immersion in water (Bar = 50 μm). **H,** SEM photo of a single encysted zoospore that germinated and directly penetrated the epidermis of a cucumber fruit (Bar = 4 μm). **I,** Typical amphigynous oospore (Bar = 10 μm). **J,** A germinating oospore with multiple germ tubes and a terminal sporangium (Bar = 100 μm).

caused by *P. capsici*. Rotation to nonsusceptible hosts, in conjunction with cultural and chemical control strategies, have not provided economic control. Correspondence with other vegetable pathologists suggests that this phenomenon is not confined to Michigan, and a similar increase in control failures due to blight by *P. capsici* is being reported throughout the United States.

Investigation of the inheritance of MS demonstrated that MS is inherited as a single incompletely dominant gene unlinked to CT. In 1998, all six possible MS × CT combinations were present in single fields and insensitivity to mefenoxam was common in Michigan. Typical amphigynous oospores were observed in *P. capsici*-infected cucurbit fruit from multiple locations, and oospore progeny from a single naturally infected fruit showed segregation for MS and CT. These findings strongly support the hypothesis that sexual reproduction is occurring in the field, and also suggest that sexual recombination may directly generate progeny fully insensitive to mefenoxam. Tracking a single mefenoxam insensitive population over 2 years in the absence of mefenoxam selection pressure suggests that costs associated with mefenoxam insensitivity are minimal.

Estimates of average heterozygosity and polymorphism indicate surprisingly high levels of gene and genotypic diversity in all the populations of *P. capsici* analyzed. Tracking a single population through an entire growing season showed that asexual reproduction plays a significant role in disease development within a single season. Sampling single fields over consecutive years suggested that clones do not survive Michigan winters and that oospores are the primary survival propagule. Estimation of fixation indices for samples from the same site over consecutive years suggested that there was not a significant reduction in genetic diversity between growing seasons. This implies that populations are large enough to withstand dramatic effects of genetic drift. Cluster analysis revealed unambiguous groups corresponding to geographical locations with regional populations showing more similarity overall than populations from different regions. Population pairwise fixation indices corroborated this finding. The estimated overall fixation index and AMOVA are in agreement with both, suggesting that most (approx 60%) of the total genetic variability in Michigan is found within any one population, but that a relatively large component (40%) of genetic variability is found among populations.

Recommendations based on our findings are as follows: (i) the fungicide mefenoxam may be of limited usefulness because insensitivity appears to be selected for rapidly and is unlikely to decrease when mefenoxam selection pressure is removed; (ii) fields with epidemics are likely to harbor oospores for an extended amount of time (at least 5 years), and this factor must be considered before replanting to susceptible hosts; and (iii) factors that may contribute to the introduction of *P. capsici* into uninfested fields (e.g., drainage ditches between farms, irrigation ponds, and the dumping of culls) need to be considered and if possible avoided, because once an epidemic is established we have found no evidence that the population will become extinct in an agriculturally meaningful time period.

From an evolutionary perspective, it is clear that *P. capsici* has successfully colonized a number of geographical locations in Michigan and that each of the populations sampled thus far have similarly high levels of genetic variability. The genetic stability of single populations over multiple years, the high fixation indices between even geographically close populations (1 km), and the clear structuring based on UPGMA cluster analysis all suggest that long-distance dispersal of inoculum is not common and that geographically isolated populations are also genetically isolated. It appears that the sexual stage of the *P. capsici* life cycle plays a significant role in survival as well as maintaining both genic and genotypic diversity, and has likely played a key role in the evolution of mefenoxam insensitivity. The combination of high levels of genetic variability, thick-walled oospores, and polycyclic asexual disease development make *P. capsici* a formidable pathogen (Fig. 1). This work underscores the need for management strategies aimed at preventing the spread of *P. capsici* to uninfested field sites and suggests that management strategies aimed at limiting spread within a single season may be the only option for growers with *P. capsici*-infested fields.

ACKNOWLEDGMENTS

This work was funded by the Michigan Agricultural Experiment Station, Michigan State University Extension, Michigan Department of Agriculture, Michigan Farm Bureau (GREEEN cooperative), Pickle and Pepper Research Committee, Pickle Packers International, Inc., and the Pickle Seed Research Fund, Pickle Packers International. We thank M. Bour, C. Hunter, J. Jabara, P. Tumbalam, E. Webster, and J. Woodworth for competent laboratory assistance. K. Lamour thanks his Ph.D. committee members A. Jarosz, R. Hammerschmidt, and F. Trail for guidance and extends sincere thanks to M. Hausbeck for fulfilling her role as mentor in an exemplary manner.

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Worksheet 3-A(20). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative Use additional pages as need.

For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third researcl the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For t... alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: General IPM   Study: UNEP 1998, B-91, B-94, B-285, B-288, B-38, B-93, B-286,

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted X
1b. Township caps ____
1c. Alternative not acceptable in consuming country ____
1d. Other (Please describe) ____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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ID# ____________

Worksheet 3-A(20). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes X No ____

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ____________

3. Publication and Date of Publication ____________

4. Location of research study ____________

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

   General IPM

6. Was crop yield measured in the study? Yes ____ No ____

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?

   I would not expect similar results because Michigan solanaceous crop growers use extensive IPM practices, but have severe disease due to Phytophthora crown and fruit rot. Some of the IPM practices that growers use include crop rotation, raised beds, mulch, trickle irrigation, and fungicide sprays. These practices, even when used in combination, have not been adequate to manage *Phytophthora capsici*.

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ID#________

Worksheet 3-A(20)(b). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. <u>You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative</u> Use additional pages as need.

<u>For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed</u>. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

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Use additional pages as needed.

Alternative: General IPM

Study: The spatiotemporal genetic structure of *Phytophthora capsici* in Michigan and implications for disease management.

Section I. Initial Screening on Technical Feasibility of Alternatives

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1a. Full use permitted ___X___
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1c. Alternative not acceptable in consuming country ________
1d. Other (Please describe) ________

____

____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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Worksheet 3-A(20)(b). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. **Is the study on EPA's website?** Yes ____ No X

   1a. **If not on the EPA website, please attach a copy.**

2. **Author(s) or researcher(s)** K.H. Lamour
   M.K. Hausbeck

3. **Publication and Date of Publication** Phytopathology 92:681-684, 2002

4. **Location of research study** Michigan, USA

5. **Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.**
   General IPM

6. **Was crop yield measured in the study?** Yes ____ No X

7. **Describe the effectiveness of the alternative in controlling pests in the study.**
   Growers in Michigan follow recommended disease management strategies, including water management, planting on raised beds, rotation to nonsusceptible hosts and the use of fungicidés, and still suffer losses from *Phytophthora capsici*.

8. **Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?**
   The results of this study are directly applicable, since the research was conducted in Michigan, USA.

OMB Control # 2060-0482

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Accepted for publication 12 February 2002.

Root, crown, and fruit rot caused by *Phytophthora capsici* Leonian is a limiting factor for the production of peppers, tomatoes, and cucurbit crops in Michigan and the United States. Like many species in the genus *Phytophthora*, *P. capsici* has the potential for rapid polycyclic disease development from a limited amount of initial inoculum (6). *P. capsici* produces caducous sporangia that can be spread by wind-blown rain or release 20 to 40 motile zoospores in the presence of free water. The polycyclic phase of disease development is thought to be driven primarily by asexual spore dispersal at a local scale (within and down rows). Sexual reproduction requires both the A1 and A2 compatibility types (CTs) and results in the production of thick-walled oospores. Oospores are thought to serve as the primary survival structure outside of host tissue.

Recommended disease management strategies stress the importance of avoiding excess water in the plant rhizosphere by using well-drained fields, conservative irrigation, and planting on raised beds. Additional recommendations include rotation to nonsusceptible hosts for at least 2 years and the use of fungicides. The phenylamide fungicide (PAF) mefenoxam is a systemic compound with high activity against *P. capsici* and has been used by growers throughout the United States to control *P. capsici*. Insensitivity to PAF has been reported for a number of other oomycetous organisms (*Bremia lactucae*, *P. infestans*, and *P. sojae*, etc.) and appears to be conferred by a single incompletely dominant gene of major effect (1). Growers in Michigan practicing 2+-year rotation in well-drained fields using an array of fungicidal management tools have experienced significant losses to *P. capsici*. Michigan is the number one producer of cucumbers for pickling in the United States and it was at the request of grower groups associated with this industry that research into the epidemiology and reproductive biology of *P. capsici* on cucurbit hosts was initiated.

Although many researchers cite oospores as the most likely propagule for survival outside of host tissue, there have been very few investigations specifically aimed at determining the impact of sexual reproduction in natural populations of *P. capsici*. Our hypothesis was that the sexual stage may play an important role not only in survival but also in the adaptation of *P. capsici* populations to environmental stresses (e.g., fungicides). Our goal was to perform a comprehensive investigation of the phenotypic and genetic diversity present in *P. capsici* populations from the major vegetable production regions of Michigan, with the implicit intention of addressing questions concerning epidemiology, reproductive biology, and the durability of currently recommended management strategies.

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Publication no. P-2002-0325-02O

METHODOLOGY

Isolate collection and maintenance. Sampling of diseased fields began at the end of the 1997 growing season and continued through September 2000. In all cases, fields were sampled on a grid with quadrants varying from 40 m^2 to 12 km^2. A limited number of isolates were collected in 1997. In 1998, the strategy was to collect as many samples from as many fields as possible. This strategy was modified in 1999 and 2000 to focus on specific fields. Isolations from diseased plants were made onto selective media and single zoospore cultures were generated according to standard single sporing techniques (3). Isolates were placed into long-term storage (15°C) using a hemp seed/sterile water technique.

Phenotypic characterization. Single zoospore isolates were screened for CT using known A1 and A2 isolates. In vitro screening techniques published for other *Phytophthora* species for assessing sensitivity to mefenoxam were compared and a novel, simple, high dose screen using 100 ppm of mefenoxam-amended V8 agar was found to separate field isolates into three modal distributions that appeared consistent with the expectations of a single incompletely dominant gene governing mefenoxam insensitivity (e.g., sensitive, intermediately sensitive, and fully insensitive). These putative mefenoxam sensitivity (MS) groupings were tested by performing a series of crosses and testing whether the observed progeny sets met the expectations for Mendelian inheritance of a single incompletely dominant gene controlling insensitivity to mefenoxam. Sexual crosses were conducted on unclarified V8 agar plates and incubated for 3 months in the dark. Individual germinated oospores were recovered after 3 months using previously published techniques (2).

The efficacy of this in vitro mefenoxam screening technique was further tested in pumpkin seedlings using progeny from a cross between parents intermediately sensitive to mefenoxam. Nine isolates from each of the three MS categories were screened for pathogenicity on untreated seedlings. Single sensitive, intermediately sensitive, and fully insensitive isolates were then placed onto the unwounded surface of plants treated with either a field rate of mefenoxam, three times the field rate, or distilled water. Lesion diameters on seedling stems were measured after 4 days.

Genetic characterization. Single zoospore isolates were grown in antibiotic-amended V8 broth for 3 days at room temperature. Mycelial mats were washed, frozen, lyophilized, and ground with a sterile mortar and pestle. DNA was extracted with either a Qiagen Dneasy extraction kit (Qiagen, Valencia, CA) or via a cetyltrimethylammonium bromide (CTAB) procedure. A variety

of methods for generating molecular markers were tested for efficacy including isozyme, random amplified polymorphic DNA, and amplified fragment length polymorphism (AFLP). The AFLP technique resulted in a large number of reproducible markers and was chosen to characterize samples of *P. capsici* from Michigan. The AFLP technique involves cutting genomic DNA with moderately rare cutting (*Eco*RI) and frequent cutting (*Mse*I) restriction enzymes, while concomitantly ligating synthetic adaptor fragments of DNA to the sticky ends created by the restriction enzymes (7). The result is a large number of DNA fragments that have ends with known DNA sequences. Amplification of fragment subsets (termed fingerprints) can be accomplished using polymerase chain reaction (PCR) primers complementary to the adaptor sequences with additional "selective" nucleotides. Changing the amount and type of selective nucleotides results in different subsets or fingerprints. Stringent PCR cycling parameters (touchdown technique) are used to ensure the fidelity of the reaction. For the analysis summarized here, adaptor sequences and fluorescent labeled selective primers were purchased as a kit through Perkin-Elmer ABI (Applied Biosystems, Foster City, CA). Using this system, AFLP fragments were resolved on a polyacrylamide gel by an ABI 377 gene sequencer. Fluorescent labels were excited by a laser and band emissions were analyzed in the form of an electropherogram where peaks represent individual bands. The sizing of fragments was particularly robust because a DNA ladder was loaded with every sample into the gel. To test for the reproducibility of fingerprints, DNA was extracted from a single isolate on three separate occasions approximately 3 months apart and subjected to the aforementioned protocol.

Data analysis. Isolates with identical multilocus AFLP fingerprints were considered to be members of the same clonal lineage and only a single representative was used for analysis. Because AFLP markers can only be scored confidently for presence (1) or absence (0), allele frequencies were estimated based on the assumption that populations under investigation meet the criterion for Hardy-Weinberg equilibrium, and that loci have only one "present" allele. The term population refers to all samples taken from a single field during a single year.

Genetic diversity within single populations was assessed by calculating the average number of polymorphic bands and estimating the average heterozygosity. Fixation indices were calculated according to methods of Weir and Cockerham (8) for populations from the same site over multiple years and among populations in Michigan using the program tools for population genetic analysis (TFPGA) (M. P. Miller, Northern Arizona University, Flagstaff). Confidence intervals for F statistics at the 95% confidence level were generated by bootstrapping at 1,000 iterations. The program NTSYS-pc version 2.02k (Exeter Software, Setauket, NY) was used to construct a similarity matrix from the presence/absence (1/0) data. Cluster analysis using the unweighted pair group with arithmetic averages (UPGMA) method was performed on the matrix and a tree was generated to give a visual representation of isolate similarity. Excoffier's ARLEQUIN program (L. Excoffier, University of Geneva) was used to assess population differentiation using a phenetic approach termed analysis of molecular variance (AMOVA), which allows for total genetic variation to be partitioned within and among populations using a classical analysis of variance (ANOVA).

RESULTS

Phenotypic results. Five isolates were recovered in 1997 from five different farms (four A1 and one A2 CT). One isolate was fully insensitive to mefenoxam, whereas the other four were fully sensitive. These findings prompted the extensive sampling conducted in 1998 in which 523 isolates (473 from cucurbits and 30 from bell pepper) were collected from 14 farms. A frequency histogram plotting percent growth of control on 100 ppm of mefenoxam-amended media versus number of isolates revealed a trimodal distribution (3). Putative MS categories were assigned based on these groupings with sensitive (S) <30% growth of control, intermediately sensitive (IS) between 30 and 90% growth of control, and insensitive (I) >90% growth of control. In vitro crosses between isolates representative of the different putative sensitivity categories (S × S, I × S, IS × S, and IS × IS) resulted in progeny sets not significantly different than expected for insensitivity inherited as a single incompletely dominant gene unlinked to CT ($P = 0.05$) (3). In 1998, 55% of the isolates were sensitive to mefenoxam, 32% were intermediately sensitive, and 13% were fully insensitive to mefenoxam. A1 and A2 CTs were recovered in a ratio of approximately 1:1 in 8 of the 14 farms. Oospores were detected in naturally diseased cucurbit fruit from four farms, and 223 oospore progeny were recovered and germinated from a single diseased cucumber. All six possible MS × CT combinations were detected in this naturally occurring oospore progeny set (3).

In planta studies using sensitive, intermediately sensitive, and fully insensitive *P. capsici* isolates supported the in vitro screening categories, with sensitive isolates causing no disease on mefenoxam-treated plants, intermediately sensitive isolates being slowed by mefenoxam, and fully insensitive isolates showing no difference in the ability to colonize host tissue between treated and untreated plants at three times the field rate. All the progeny isolates were pathogenic on untreated pumpkin plants (K. H. Lamour and M. K. Hausbeck, *unpublished data*).

Sixty-three mefenoxam insensitive (18% intermediate and 82% fully insensitive) isolates were recovered from a single southwest Michigan field in 1998. Field experiments were conducted in this field during 1999 and 2000, testing alternative cultural control strategies, and no mefenoxam was applied. Two hundred isolates were recovered from this site over the course of the 1999 season and 34 isolates at the beginning of the 2000 season. Of the 200 isolates recovered in 1999 from this field, 141 had unique AFLP genotypes. Seventy percent of these were fully insensitive to mefenoxam, 28% were intermediately sensitive, and 2% were sensitive. In 2000, 15% of the isolates were intermediately sensitive and 85% were fully insensitive. A single fully insensitive clonal lineage rose in frequency over the course of the 1999 season and comprised 20% of the total number of samples recovered (4).

During 1999 and 2000, approximately 2,500 isolates were recovered from farms in Michigan. Both the A1 and A2 CTs were present in every field sampled, and mefenoxam insensitivity was detected in the majority of farms that had a history of mefenoxam use.

Genetic results. Nine populations from the four major vegetable production areas of Michigan were analyzed with the AFLP procedure ($N = 641$). AFLP analysis resolved a total of 94 clearly discernable markers when considering all the isolates together. No single isolate or group of isolates from a single location contained all 94 markers. The total number of AFLP loci in a single population ranged from 68 to 80. Seventeen (18%) fragments were fixed for the present state across all populations, 12 (13%) fragments were polymorphic in all populations, and 65 (69%) were fixed for presence or absence in some populations and polymorphic in others. The number of polymorphic bands within a single population ranged from 37 to 46 with estimated heterozygosities ranging from 0.18 to 0.22. Clonal reproduction was significant within single fields over the course of the growing season. For example, genotypic diversity in a single field ranged from 100% at the beginning of the growing season (seedling stage) to <30% at the time cucurbit fruit were ready for harvest (4). When considering all nine populations, genotypic diversity ranged from 42 to 96% with an average of 74% of the isolates in any sample set having unique genotypes. Although clonal reproduction was significant within single fields within years, no clones were recovered from single fields between years or among fields separated by at least 1 km. Fixation indices (ϕ_{ST}) between the

populations sampled on consecutive years were very close to zero, indicating that gene diversity was not measurably impacted by genetic drift (5). The overall estimated ϕ_{ST} for populations from different locations was 0.35, indicating that approximately 35% of the total genetic diversity present in Michigan *P. capsici* populations is found among populations and 65% is found within any one population. AMOVA partitioned genetic diversity among (40%) and within (60%) populations. The similarity tree based on UPGMA cluster analysis clearly showed that isolates from the same site sampled over years branched from the same node, with no clustering of isolates based on the year of sampling. Cluster analysis also clearly showed that populations separated geographically branched from population-specific nodes (5).

DISCUSSION

During the past 10 years, Michigan has experienced a steady increase in the incidence of root, fruit, and crown rot on cucurbits

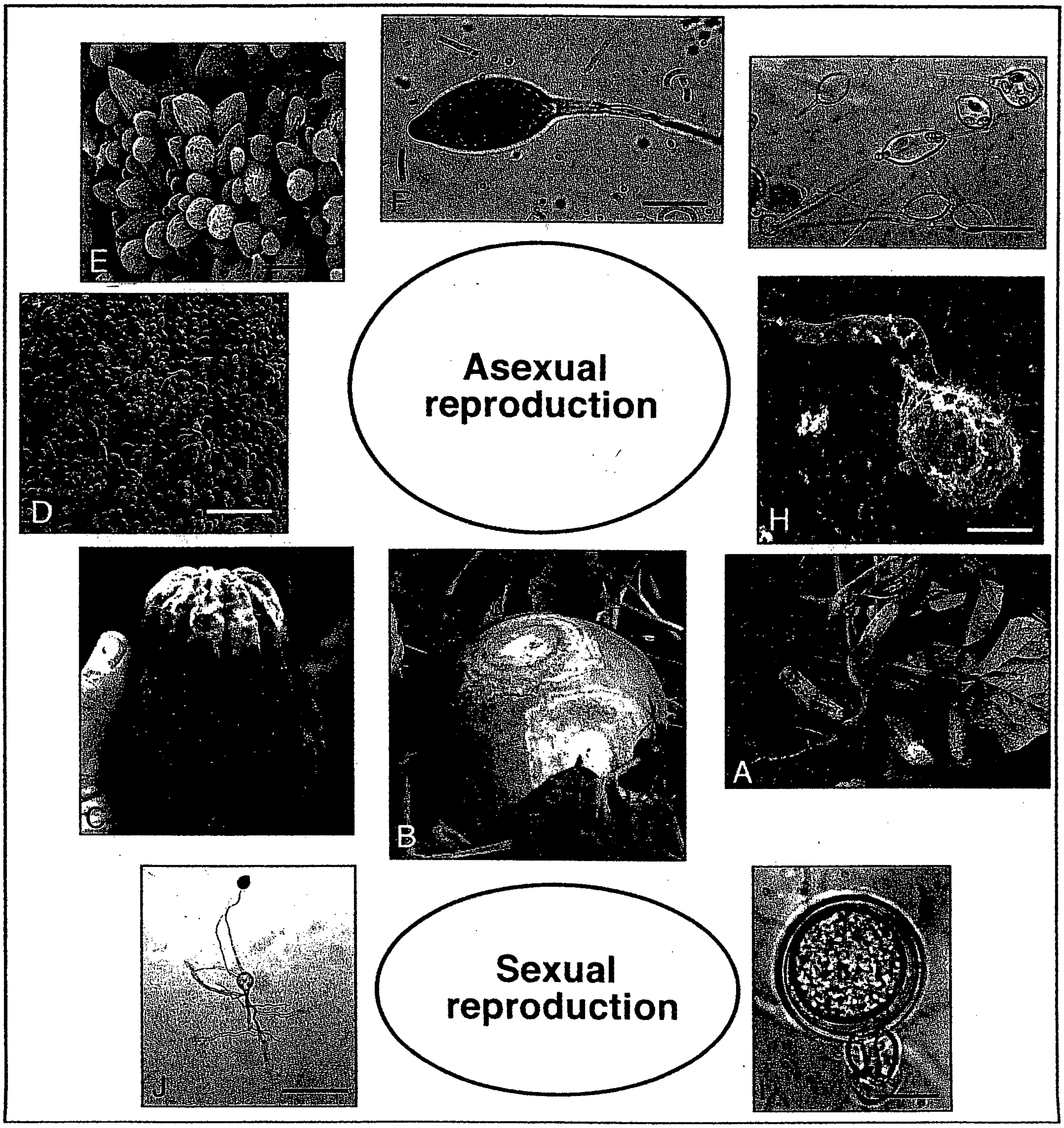

Fig. 1. Spore types and signs of infection caused by *Phytophthora capsici* on cucurbit fruit: **A,** infected cucumber, **B,** pumpkin, and **C,** acorn squash fruit. **D,** Scanning electron microscope (SEM) photo of an infected cucumber showing tufts of sporangia produced on the surface of the fruit (Bar = 300 μm). **E,** Close-up of a single tuft of sporangia (Bar = 30 μm). **F,** Typical papillate sporangium with a long pedicel (Bar = 20 μm). **G,** Zoospores exiting sporangia after immersion in water (Bar = 50 μm). **H,** SEM photo of a single encysted zoospore that germinated and directly penetrated the epidermis of a cucumber fruit (Bar = 4 μm). **I,** Typical amphigynous oospore (Bar = 10 μm). **J,** A germinating oospore with multiple germ tubes and a terminal sporangium (Bar = 100 μm).

caused by *P. capsici*. Rotation to nonsusceptible hosts, in conjunction with cultural and chemical control strategies, have not provided economic control. Correspondence with other vegetable pathologists suggests that this phenomenon is not confined to Michigan, and a similar increase in control failures due to blight by *P. capsici* is being reported throughout the United States.

Investigation of the inheritance of MS demonstrated that MS is inherited as a single incompletely dominant gene unlinked to CT. In 1998, all six possible MS × CT combinations were present in single fields and insensitivity to mefenoxam was common in Michigan. Typical amphigynous oospores were observed in *P. capsici*-infected cucurbit fruit from multiple locations, and oospore progeny from a single naturally infected fruit showed segregation for MS and CT. These findings strongly support the hypothesis that sexual reproduction is occurring in the field, and also suggest that sexual recombination may directly generate progeny fully insensitive to mefenoxam. Tracking a single mefenoxam insensitive population over 2 years in the absence of mefenoxam selection pressure suggests that costs associated with mefenoxam insensitivity are minimal.

Estimates of average heterozygosity and polymorphism indicate surprisingly high levels of gene and genotypic diversity in all the populations of *P. capsici* analyzed. Tracking a single population through an entire growing season showed that asexual reproduction plays a significant role in disease development within a single season. Sampling single fields over consecutive years suggested that clones do not survive Michigan winters and that oospores are the primary survival propagule. Estimation of fixation indices for samples from the same site over consecutive years suggested that there was not a significant reduction in genetic diversity between growing seasons. This implies that populations are large enough to withstand dramatic effects of genetic drift. Cluster analysis revealed unambiguous groups corresponding to geographical locations with regional populations showing more similarity overall than populations from different regions. Population pairwise fixation indices corroborated this finding. The estimated overall fixation index and AMOVA are in agreement with both, suggesting that most (approx 60%) of the total genetic variability in Michigan is found within any one population, but that a relatively large component (40%) of genetic variability is found among populations.

Recommendations based on our findings are as follows: (i) the fungicide mefenoxam may be of limited usefulness because insensitivity appears to be selected for rapidly and is unlikely to decrease when mefenoxam selection pressure is removed; (ii) fields with epidemics are likely to harbor oospores for an extended amount of time (at least 5 years), and this factor must be considered before replanting to susceptible hosts; and (iii) factors that may contribute to the introduction of *P. capsici* into uninfested fields (e.g., drainage ditches between farms, irrigation ponds, and the dumping of culls) need to be considered and if possible avoided, because once an epidemic is established we have found no evidence that the population will become extinct in an agriculturally meaningful time period.

From an evolutionary perspective, it is clear that *P. capsici* has successfully colonized a number of geographical locations in Michigan and that each of the populations sampled thus far have similarly high levels of genetic variability. The genetic stability of single populations over multiple years, the high fixation indices between even geographically close populations (1 km), and the clear structuring based on UPGMA cluster analysis all suggest that long-distance dispersal of inoculum is not common and that geographically isolated populations are also genetically isolated. It appears that the sexual stage of the *P. capsici* life cycle plays a significant role in survival as well as maintaining both genic and genotypic diversity, and has likely played a key role in the evolution of mefenoxam insensitivity. The combination of high levels of genetic variability, thick-walled oospores, and polycyclic asexual disease development make *P. capsici* a formidable pathogen (Fig. 1). This work underscores the need for management strategies aimed at preventing the spread of *P. capsici* to uninfested field sites and suggests that management strategies aimed at limiting spread within a single season may be the only option for growers with *P. capsici*-infested fields.

ACKNOWLEDGMENTS

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Worksheet 3-A(21). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative. Use additional pages as need.

For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questio

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

- **Use additional pages as needed.**

Alternative: **Grafting, Resistant Rootstock, Plant Breeding**

Study: **UNEP 1995, UNEP 1998, UNEP 2000, A-76, B-46, B-47, B-83, B-94, B-281, B-282, D-91, D-105, D-109, B-87, B-287**

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted ___X___
1b. Township caps ______
1c. Alternative not acceptable in consuming country ______
1d. Other (Please describe) ______

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

Worksheet 3-A(21). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes __X__ No ____

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ____________

3. Publication and Date of Publication ____________

4. Location of research study ____________

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

   Grafting, Resistant Rootstock, Plant Breeding

6. Was crop yield measured in the study? Yes ____ No ____

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?

   The results of the study do not readily apply to solanaceous crop production in Michigan. The study focuses on fruit trees and grapes for control of nematodes and soil-borne pathogens. Grafting solanaceous crops onto resistant rootstock will not solve the problem of fruit rot. Currently, resistance has not been identified.

For EPA Use Only
ID# ________________

Worksheet 3-A(22). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative. Use additional pages as need.

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When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or moi research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Res Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

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The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Organic Amendments, Compost **Study:** UNEP 1998, B-281, B-283, B-40, B-87, B-287

Section I. Initial Screening on Technical Feasibility of Alternatives

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   1a. Full use permitted ____X____
   1b. Township caps ________
   1c. Alternative not acceptable in consuming country ________
   1d. Other (Please describe) ________

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If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

For EPA Use Only
ID# ______________

Worksheet 3-A(22). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. **Is the study on EPA's website?** **Yes** X **No** ______

    1a. **If not on the EPA website, please attach a copy.**

2. **Author(s) or researcher(s)** ______________________________

3. **Publication and Date of Publication** ______________________________

4. **Location of research study** ______________________________

5. **Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.**

    Organic Amendments, Compost

6. **Was crop yield measured in the study?** **Yes** ______ **No** ______

7. **Describe the effectiveness of the alternative in controlling pests in the study.**

    ____

8. **Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?**

    The studies include root-knot nematodes, *Fusarium* and *Verticillium*, and do not account for *Phytophthora capsici*, which is Michigan's primary problem. Therefore, the results of these studies do not reflect what would be expected to occur in Michigan, USA.

Worksheet 3-A(23). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative. Use additional pages as need.

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In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Planting Time **Study: UNEP 1998, B-41**

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted ___X___
1b. Township caps ______
1c. Alternative not acceptable in consuming country ______
1d. Other (Please describe) ______

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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ID# ______________

Worksheet 3-A(23). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes X No ____

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ______________

3. Publication and Date of Publication ______________

4. Location of research study ______________

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

   Planting Time

6. Was crop yield measured in the study? Yes ____ No ____

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?

   Phytophthora capsici is a problem at all times of the year, and cannot be avoided by altering the planting time.

OMB Control # 2060-0482

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ID# ____________

Worksheet 3-A(24). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. <u>You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative</u> Use additional pages as need.

<u>For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed</u>. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Plowing and Tillage **Study:** UNEP 1998, B-41

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted ___X___
1b. Township caps ______
1c. Alternative not acceptable in consuming country ______
1d. Other (Please describe) ______

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

For EPA Use Only
ID# ____________

Worksheet 3-A(24). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes X No ____

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ____________

3. Publication and Date of Publication ____________

4. Location of research study ____________

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

   Plowing and Tillage

6. Was crop yield measured in the study? Yes ____ No ____

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. *Would you expect similar results? Are there other factors that would affect your adoption of this tool?*

   Michigan growers would not expect similar results, since the cited study did not include *Phytophthora capsici*. The cited study does not list pathogens which are primary problems in Michigan, USA.

For EPA Use Only
ID# ____________

Worksheet 3-A(25). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative Use additional pages as need.

For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
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(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be con scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Resistant Cultivars **Study:** UNEP 1998, B-83, B-87, B-94, B-282, B-283, B-284, B-288, B-46, B-287

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted X
1b. Township caps ____
1c. Alternative not acceptable in consuming country ____
1d. Other (Please describe) ____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

Worksheet 3-A(25). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes __X__ No ____

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ____________

3. Publication and Date of Publication ____________

4. Location of research study ____________

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.
   Resistant Cultivars

6. Was crop yield measured in the study? Yes ____ No ____

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?
   The comments in these reports do not state that genetic resistance to *Phytophthora capsici* exists.

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ID# ____________

Worksheet 3-A(25)(b). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. <u>You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative</u> Use additional pages as need.

<u>For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed</u>. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Resistant Cultivars **Study:** Pepper Phytophthora resistant variety Test, Martino Farm, Vineland, NJ, 2000.

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted X
1b. Township caps ____
1c. Alternative not acceptable in consuming country ____
1d. Other (Please describe) ____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

For EPA Use Only
ID# ____________

Worksheet 3-A(25)(b). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. **Is the study on EPA's website?** Yes X No ____

   1a. **If not on the EPA website, please attach a copy.**

2. **Author(s) or researcher(s)** S.A. Johnston, S.A. Garrison, M.L. Fogg, M.D. Zimmerman, W.L. Kline

3. **Publication and Date of Publication** Rutgers University report, 2000

4. **Location of research study** New Jersey, USA

5. ***Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.***
   Resistant varieties of bell and non-bell pepper types.

6. **Was crop yield measured in the study?** Yes X No ____

7. **Describe the effectiveness of the alternative in controlling pests in the study.**
   All pepper varieties suffered blight due to *Phytophthora capsici,* with even the most resistant variety showing 28% blight by the end of the study.

8. **Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?**
   Michigan growers would expect to see similar results, because the cultural practices and environmental conditions are comparable to those in New Jersey.

PEPPER (*Capsicum sp.*)
Blight, *Phytophthora capsici*

S.A. Johnston, S.A. Garrison, M.L. Fogg,
M.D. Zimmerman & W.L. Kline

PEPPER PHYTOPHTHORA RESISTANT VARIETY TEST, MARTINO FARM, VINELAND, NJ, 2000: The experiment was conducted on the Robert Martino Farm in Vineland, NJ (Cumberland County). The field has been in continuous pepper production for greater than 25 years, and is naturally infested with *Phytophthora capsici*. On 2 Jun, the field was prepared for transplanting peppers by making ridges. Bare-rooted pepper transplants were mechanically transplanted onto the ridges with a commercial transplanter. Plots consisted of a single 13 ft long row that contained 8 plants, and rows were spaced 3 ft apart. Varieties were replicated 4 times in a randomized complete block design. Rainfall (June 6 – August 24) at Rutgers Agricultural Research & Extension Center (located approximately 20 miles from site of pepper experiment) was 3.6 in. in Jun; 3.0 in. in Jul; and 3.5 in. in Aug [June rainfall (in inches): 6/6=0.46; 6/12=0.27; 6/14=0.11; 6/15=0.06; 6/16=0.02; 6/18=0.45; 6/22=0.31; 6/26=1.12; 6/28=0.70; 6/29=0.04; 6/30=0.08; July rainfall (in inches): 7/3=0.20; 7/4=0.34; 7/13=0.01; 7/14=0.04; 7/15=0.59; 7/17=0.01; 7/19=0.39; 7/20=0.25; 7/22=0.04; 7/24=0.03; 7/26=0.98; 7/27=0.08; 7/30=0.02; and August rainfall (in inches): 8/3=0.09; 8/4=0.11; 8/8=0.02; 8/9=0.26; 8/11=0.41; 8/12=0.34; 8/13=0.02; 8/14=1.85; 8/18=0.38]. Plots were evaluated for the incidence of Phytophthora blight 10, 17 and 24 Aug. Plots were harvested on 31 Aug, and marketable fruit were weighed for yield determinations.

Phytophthora began to appear in the field in late Jun, and the majority of plants in the susceptible varieties were infected by the end of Aug. Heavy rainfalls in early-mid Aug resulted in a rapid increase in disease incidence in susceptible varieties. 'Paladin' and 'Aristotle' consistently had significantly fewer infected plants compared to the susceptible check variety, 'Cherry'. 'LBV' had significantly fewer infected plants than the check at the first two evaluation dates only, and 'HMX9640' had significantly fewer infected plants than the check only at the 17 Aug evaluation date. Only 'Paladin' produced a significantly greater yield than the check.

		% Blight*			
Variety	Type	10 Aug	17 Aug	24 Aug	Yield (Bu/A)
Cherry............	Non-bell	27.3a	47.3ab	74.4ab	120.6b-d
Paladin.............	Bell	5.2cd	5.2fg	28.3d	312.1a
Aristotle...........	Bell	7.5b-d	24.1c-f	37.0d	91.0cd
BHN-1...........	Bell	19.8a-c	29.5b-e	60.7b	72.8cd
HMX9640.......	Bell	18.4a-d	20.9d-f	72.0ab	75.5cd
AZ8...............	Non-bell	32.6a	53.4a	77.4ab	68.9cd
AZ9...............	Non-bell	23.9a-c	49.7ab	72.3ab	170.0bc
AZ20.............	Non-bell	24.4ab	33.8a-d	79.9ab	239.3ab
LBV...............	Non-bell	0.0d	10.4e-g	61.7ab	3.7d
LIH................	Non-bell	25.4ab	35.7a-d	59.0bc	3.3d
LJM...............	Non-bell	28.1a	41.4a-c	82.5a	2.5d
LJE................	Non-bell	5.2cd	0.0g	38.1cd	12.4d
LSD (P≤0.05)		18.8	20.25	21.4	132.5

* Data transformed using arcsine square root

Worksheet 3-A(25)(c). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative. Use additional pages as need.

For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Resistant Cultivars **Study:** Pepper Phytophthora resistant variety test, 2000.

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted ___X___
1b. Township caps ______
1c. Alternative not acceptable in consuming country ______
1d. Other (Please describe) ______

____
____
____
____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

For EPA Use Only
ID# __________

Worksheet 3-A(25)(c). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. **Is the study on EPA's website?** **Yes** X **No** ____

   1a. **If not on the EPA website, please attach a copy.**

2. **Author(s) or researcher(s)** S.A. Johnston, S.A. Garrison, M.L. Fogg, M.D. Zimmerman, W.L. Kline

3. **Publication and Date of Publication** Rutgers University report, 2000

4. **Location of research study** New Jersey, USA

5. **Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.**
   Resistant varieties of bell and non-bell pepper types.

6. **Was crop yield measured in the study?** **Yes** X **No** ____

7. **Describe the effectiveness of the alternative in controlling pests in the study.**
   All pepper varieties suffered blight due to *Phytophthora capsici,* with even the most resistant variety showing 47% blight by the end of the study.

8. **Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?**
   Michigan growers would expect to see similar results, because the cultural practices and environmental conditions are comparable to those in New Jersey.

PEPPER (*Capsicum annuum*)
Blight; *Phytophthora capsici*

S.A. Johnston, S.A. Garrison, M.L. Fogg,
M.D. Zimmerman & W.L. Kline

PEPPER PHYTOPHTORA RESISTANT VARIETY TEST, 2000: The experiment was conducted in a field that produced peppers in 1999 with 100% of plants infected with Phytophthora blight at the end of the season. The field (Aura loam, pH 6.3) was located at the Rutgers Agricultural Research & Extension Center in Upper Deerfield Township. On 12 Mar, the field was 'V-ripped', and 24 Apr, fertilizer (400 lb/A of calcium nitrate – 62 lb nitrogen/A) was applied and incorporated. On 27 Apr, Command 3ME (21 fl.oz./A) + Devrinol 50DF (3 lb/A) were applied and lightly incorporated for weed control. On 28 Apr, black polyethylene mulch and Netafim drip irrigation tubing was laid onto the field in flat culture. On 2 Jun, Dual II Magnum 7.6E (1.5 pt/A) + Gramoxone Extra 2.5E (2 pt/A) was applied to the row middles for weed control. On 5 Jun, pepper varieties were transplanted into the field with a Kennco water wheel transplanter. Plots consisted of a 15-ft-long double row of peppers (10 plants/row; 20 plants/plot). Varieties were replicated 4 times in a randomized complete block design. Supplemental soil fertility (30 lb/A of N-P-K, Blu-Gro ~~LC~~ with minor elements, Plant Food Company, Inc., Cranbury, NJ) was applied via injection through the drip irrigation system on 22 Jun, 13 Jul and 3 Aug. Rainfall was 3.6 in. in Jun, 3.0 in. in Jul, 3.7 in. in Aug and 4.1 in. in Sep. Tensiometers were placed within two randomly selected rows to measure soil moisture 6 and 12 in. below the mulch. Whenever tensiometer readings registered 50cb or greater soil tension, supplemental irrigation was applied via the drip irrigation system. Plots were visually evaluated for the incidence of Phytophthora blight on 30 Jun, 5, 20 Jul, 2, 18 Aug and 2 Oct. Plots were harvested on 10 Aug.

Since the field was planted to peppers in 1999, and 100% of the plants were infected with Phytophthora blight, the early season rains and lack of raised bed culture resulted in a high incidence of disease in the field in 2000. Phytophthora blight began to appear in the field in late June, and rapidly progressed through the field with 75% of plants of the susceptible cultivar, 'Camelot', infected by early July. 'Paladin', 'Aristotle' and 'BHN-1' significantly reduced the percentage of Phytophthora blight compared the 'Camelot' at all evaluation dates until mid-August. 'BHN-1' did not significantly reduce blight compared to 'Camelot' at the last evaluation date in August; whereas, 'Paladin' and 'Aristotle' still had signficantly less blight. By early October there were no significant differences among varieties in blight. 'Paladin' was particular effective in reducing the incidence of the crown rot phase of blight; however, it was susceptible to the aerial phase of the disease which resulted in the high incidence of blight by the end of the season.

Variety	Type	% Blight*					Yield
		30 Jun	5 Jul	20 Jul	2 Aug	18 Aug	(bu/A)
Paladin	Bell	0.0c	0.0e	6.4d	21.6c	47.3c	99.4d
Aristotle	Bell	9.2bc	26.4cd	45.2c	57.8b	64.4bc	419.9ab
BHN-1	Bell	4.6c	24.8d	47.2bc	57.0b	73.4ab	401.7ab
HMX9640	Bell	21.6a	40.9b-c	66.0ab	74.7ab	85.4ab	288.5bc
Camelot	Bell	26.4a	62.3a	82.2a	86.8a	90.0a	438.5a
AZ8	Non-bell	8.9bc	45.8a-c	63.8a-c	78.0a	83.4ab	187.4cd
AZ9	Non-bell	16.8ab	49.1ab	75.8a	79.7a	90.0a	257.6c
AZ20	Non-bell	26.2a	56.0ab	71.6a	82.5a	84.3ab	173.3cd
LSD ($P \leq 0.05$)		11.4	20.2	19.0	19.8	22.4	133.9

* Data transformed using the arcsine square root.

Worksheet 3-A(26). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative. Use additional pages as need.

For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies curren... ... ... EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website and other websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and therefore no research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Soilless Culture **Study: UNEP 1998, B-44, B-83, B-282, B-287**

Section I. Initial Screening on Technical Feasibility of Alternatives

1. Are there any location-specific restrictions that inhibit the use of this alternative on your site?

1a. Full use permitted X
1b. Township caps ____
1c. Alternative not acceptable in consuming country ____
1d. Other (Please describe) ____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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Worksheet 3-A(26). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes X No ____

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ____

3. Publication and Date of Publication ____

4. Location of research study ____

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

   Soilless Culture

6. Was crop yield measured in the study? Yes ____ No ____

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?

   Soilless culture is achieved in some parts of the world through the use of volcanic gravel, and has been helpful in managing various soil-borne pathogens. This method of disease control is unproven for management of *Phytophthora capsici*, and is not feasible for Michigan growers who do not have access to volcanic gravel.

Worksheet 3-A(27). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

In this worksheet, you should address why an alternative pest management strategy on the list (see previous page) is or is not effective for your conditions. This worksheet contains 9 questions. You must complete one copy of worksheet 3-A for each research study you use to evaluate a single methyl bromide alternative Use additional pages as need.

For worksheet 3-A you must complete one worksheet for each alternative, for each research study addressed. Please number the worksheets as follows. For the same alternative, first research study, label the worksheet 3-A(1)(a). For the same alternative, second research study, label the worksheet 3-A(1)(b). For the first alternative, third research study, label the worksheet 3-A(1)(c). For the second alternative, first research study, label the worksheet 3-(A)(2)(a). For the second alternative, second research study, label the worksheet 3-(A)(2)(b).

When completing Section II, if you cite a study that is on the EPA website, you only need to complete questions 1, 5, and 8.

Summarize each of the research studies you cite in the Research Summary Worksheet.

If you prefer, you may provide the information requested in this worksheet in a narrative review of one or more relevant research reports. The narrative review must reply to Section I and questions 1 through 8 in Section II. A Research Summary Worksheet of relevant treatments should be provided for each study reviewed.

BACKGROUND

EPA must consider whether alternative pest control measures (pesticide and non-pesticidal, and their combination) could be used successfully instead of methyl bromide by crop and circumstance (geographic area.) The Agency has developed a list of possible alternative pest control regimens for various crops, which can be found at http://www.epa.gov/ozone/mbr or by calling 1-800-296-1996.

There are three major ways you can provide the Agency with proof of your investigative work.
(1) Conduct and submit your own research
(2) Cite research that has been conducted by others
(3) Cite research listed on the EPA website

Whether you conduct the research yourself or cite studies developed by others, it is important that the studies be conducted in a scientifically sound manner. The studies should include a description of the experimental methodology used, such as application rates, application intervals, pest pressure, weather conditions, varieties of the crop used, etc. All results should be included, regardless of outcome. **You must submit copies of each study to EPA** unless they are listed on the Agency website.

The Agency has posted many research studies on a variety of crops on its website and knows of more studies currently in progress. EPA will add studies to its website as they become publicly available. You are encouraged to review the EPA website websites for studies that pertain to your crop and geographic area.

In addition, EPA acknowledges that, for certain circumstances, some alternatives are not technically feasible and the research has been conducted (i.e. solarization may not be feasible in Seattle). You should look at the list of alternatives provided by the Agency and explain why they cannot be used for your crop and in your geographic area.

Use additional pages as needed.

Alternative: Substrates, Plug Plants **Study:** UNEP 1998, B-87, B-90, B-94, B-284, B-285, B-287, B-288, B-286

Section I. Initial Screening on Technical Feasibility of Alternatives

1. ***Are there any location-specific restrictions that inhibit the use of this alternative on your site?***

1a. Full use permitted X
1b. Township caps ____
1c. Alternative not acceptable in consuming country ____
1d. Other (Please describe) ____

If use of this alternative is precluded by regulatory restriction for all users covered by this application, the applicant should not complete Section II.

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Worksheet 3-A(27). Alternatives - Technical Feasibility of Alternatives to Methyl Bromide

Section II. Existing Research Studies on Alternatives to Methyl Bromide

1. Is the study on EPA's website? Yes X No ____

   1a. If not on the EPA website, please attach a copy.

2. Author(s) or researcher(s) ________________

3. Publication and Date of Publication ________________

4. Location of research study ________________

5. Name of alternative(s) in study. If more than one alternative, list the ones you wish to discuss.

   Substrates, Plug Plants

6. Was crop yield measured in the study? Yes ____ No ____

7. Describe the effectiveness of the alternative in controlling pests in the study.

8. Discuss how the results of the study apply to your situation. Would you expect similar results? Are there other factors that would affect your adoption of this tool?

   The results of these studies do not apply to the situation in Michigan, because *Phytophthora capsici* is not disseminated via seeds or transplants. The examples given in the UNEP 1998 studies include *Alternaria*, *Didymella, Fusarium oxysporum, Clavibacter michiganensis* subsp. *michiganensis, Verticillium* spp. and *Pseudomonas* spp. These examples do not apply to the situation in Michigan. Use of pathogen-free seeds and transplants is not a viable alternative for *P. capsici*.

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Worksheet 4. Alternatives - Future Research Plans

Please describe future plans to test alternatives to methyl bromide. (All available methyl bromide alternatives from the alternatives list should have been tested or have future tests planned.) There is no need to complete a separate worksheet for future research plans for each alternative - you may use this worksheet to describe all future research plans.

1. **Name of study:** Alternatives to methyl bromide for control of *Phytophthora capsici, Verticillium* spp.,and *Fusarium oxysporum* f.sp. *lycopersicae* on solanaceous crops in Michigan, USA.
2. **Researcher(s):** Dr. Mary Hausbeck
   Mr. Brian Cortright
3. **Your test is planned for:** April to October, 2003, 2004.
4. **Location:** Southwestern Michigan, USA at Michigan State University's Research and Extension Center, several plots will be placed with various commercial growers.
5. **Name of alternative to be tested:**

Multigard FFA (47, 71 gal/A)	Telone C-35 (15, 32 gal)
Multigard Protect	Chloropicrin 100%
Multigard Protect + Vapam HL (37, 56 gal/A)	Idomethane 67/33
CX-100 (applied as drip or preplant	Chicken manure composted

6. **Will crop yield be measured in the study?** Yes X No ____
   Whenever possible.
7. **If additional testing is not planned, please explain why. (For example, the available alternatives have been tested and found unsuitable, an alternative has been identified but is not yet registered for this crop, available alternatives are too expensive for this crop, etc.)**

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Worksheet 5. Additional Information

1. How will you minimize your use and/or emissions of methyl bromide?

1a. Check all methods you will use

____ Nothing
__X__ Tarpaulin (high density polyethylene)
__X__ Virtually impermeable film (VIF)
__X__ Cultural practices (please specify) rotation, raised beds, black plastic, trickle irrigation

1b. Will you use other pesticides to reduce use of methyl bromide? Yes __X__ No ____

If yes please specify. foliar fungicides including Ridomil Gold (tomato, eggplant, pepper) and Acrobat (tomato) will be used.

1c. Other non-chemical methods: (please specify):
raised beds, drip irrigation, black plastic, foliar fungicide sprays, rotation

2. Do you have access to recycled methyl bromide? Yes ____ No __X__

If yes, how many pounds? ____________ lbs.

3. Do you anticipate that you will have any methyl bromide in storage on January 1, 2005? Yes ____ No __X__

If yes, how many pounds? ____________ lbs.

4. What is the cumulative amount spent to date by the user or consortium on research to develop alternatives to methyl bromide (beginning in 1992)? $ 1.1 million

5. Other investments, if any, made to reduce your reliance on methyl bromide. Describe each investment and its associated cost.

Michigan State University's vegetable plant pathology program has made the research its top priority.

6. Identify what factors would allow you to stop or reduce your use of methyl bromide (e.g. registration of particular pesticide; completion of research plan; capital outlay).

Completion of our research plan, identification, development, and implementation of new disease managem would greatly reduce our methyl bromide use.

When do you expect these to occur? Within 5 to 10 years.

7. Range of acres farmed by growers included in this application?
(insert number of users in each category)

__4__ 0-10 acres
__8__ 10-25 acres
__6__ 25-50 acres
__5__ 50-100 acres
__6__ 100-200 acres
__1__ 200-400 acres
____ over 400 acres

Worksheet 5. Additional Information (continued)

8. **Range of square feet of the area to which applicants included in this application will apply methyl bromide?** (insert number of users in each category)

____ 0 - 5,000 sq. ft.
____ 5,001 - 10,000 sq. ft.
____ 10,001 - 20,000 sq. ft.
____ 20,001 - 40,000 sq. ft.
____ 40,001 - 80,000 sq. ft.
__4__ 80,001 - 160,000 sq. ft. (1-10 A)
__25__ over 160,000 sq. ft. (over 10 A)

I certify that all information contained in this document is factual to the best of my knowledge.

Signature Mary K. Hausbeck **Date** 9-05-02

Print Name Mary K. Hausbeck **Title** Professor

Information in this application may be aggregated with information from other applications and used by the United States government to justify claims in the national nomination package that a particular use of methyl bromide be considered "critical" and authorized for an exemption beyond the 2005 phaseout. Use of aggregate data will be crucial to making compelling arguments in favor of critical use exemptions. **By signing below**, you agree not to assert any claim of confidentiality that would affect the disclosure by EPA of aggregate information based in part on information contained in this application.

Signature Mary K. Hausbeck **Date** 9-05-02

Print Name Mary K. Hausbeck **Title** Professor

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Worksheet 6. Application Summary

This worksheet will be posted on the web to notify the public of requests for critical use exemptions beyond the 2005 phase out for methyl bromide. Therefore, this worksheet cannot be claimed as CBI.

1. **Name of Applicant:** Michigan solanaceous crop growers
2. **Location:** Michigan, USA
3. **Crop:** Solanaceous crops: including tomato, pepper, eggplant
4. **Pounds of Methyl Bromide Requested** 2005 116,408
5. **Area Treated with Methyl Bromide** 2005 2,687 acres units
6. **If methyl bromide is requested for additional years, reason for request:**

2006 113,230 lbs. Area Treated 2,636 acres units
2007 108,875 lbs. Area Treated 2,535 acres units

Place an "X" in the column(s) labeled "Not Technically Feasible" and/or "Not Economically Feasible" where appropriate. Use the "Reasons" column to describe why the potential alternative is not feasible.

Potential Alternatives	Not Technically Feasible	Not Economically Feasible	Reasons
1,3-Dichloropropene, Chloropicrin	X		Not effective.
1,3-D, Chloropicrin, Pebulate	X		Not effective.
1,3-D, Metam Sodium	X		Not effective.
Basamid	X		Not effective.
Basamid, Solarization	X		Not effective. Climate in Michigan, USA is too cold for solarization.
Metam Sodium	X		Not effective.
Metam Sodium, Crop Rotation	X		Not effective, pathogens long-lived.
Methyl Iodide	X		Not registered in USA.
Propargyl Bromide	X		Not registered in USA.
Biofumigation	X		Efficacy is not proven, requires solarization.
Solarization	X		Climate in Michigan, USA is too cold.
Solarization, Fungicides	X		Climate in Michigan, USA is too cold for solarization. Resistance has developed to registered fungicides.
Steam	X		Not technically feasible for large scale agriculture.
Biological Control	X		Efficacy is not proven.
Cover Crops, Mulching	X		Not effective; already used in commercial production.
Crop Residue Compost	X		Not tested against *Phytophthora capsici*, and efficacy can vary regionally.
Crop Rotation, Fallow	X		Not effective, pathogens long-lived, already used in commercial production.
Endophytes	X		Efficacy is not proven.
Flooding, Water Management	X		Flooding is not feasible, trickle and raised beds are used, but frequent heavy rains favor disease.
General IPM	X		Utilized by growers, but is not adequate for disease control.
Grafting, Resistant Rootstock, Plant Breeding	X		Resistant rootstock has not been identified. Would not be effective against root rot.
Organic Amendments, Compost	X		Not tested against *Phytophthora capsici*.
Planting Time	X		Not effective, *Phytophthora capsici* is a problem year-round.
Plowing and Tillage	X		Not tested against *Phytophthora capsici*.
Resistant Cultivars	X		Resistant varieties have not been identified.
Soilless Culure	X		Volcanic ash, rockwool are not viable alternatives for large-scale production in Michigan, USA.
Substrates, Plug Plants	X		Primary pathogens are not disseminated on seed or transplants.